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ABSTRACT

This laboratory manual was developed for a field-oriented high school oceanology program. The organization of the units includes a selection of supplementary activities to allow students to explore ocean studies in more depth. Included are 19 units. The units include biological oceanography, physical oceanography, and some social science topics. A suggested sequence of activities is provided. (RH)

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MARINE AND ENVIRONMENTAL

STUDIES FIELD MANUAL

A Title IV, ESEA Project

Cranston • Warwick

Rhode Island

1973

MARINE AND ENVIRONMENTAL STUDIES FIELD MANUAL

A Regional Project

Funded under Title III of the Elementary and

Secondary Education Act in the Public Schools

of Cranston and Warwick, Rhode Island

Published in September, 1973

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TABLE OF CONTENTS

ACKNOWLEDGMENT..... i

INTRODUCTION..... ii

SYLLABUS..... iii

UNIT I - BEACHES

Introduction..... 1
Beach Profile..... 2
Beach Transect..... 2
Wave Profile..... 5

UNIT II - NEKTON

Introduction..... 7
Diets of Fishes..... 9
Age Determination of Fish..... 10
Fish Tagging..... 10
Supplemental Information..... 11

UNIT III - PLANKTON

Introduction..... 12
Comparing Plankton Net Sizes..... 13
Vertical Towing..... 14
Plankton in a Cove and in an Open Bay..... 14
Effect of a Delay in Examining Samples..... 14
Effect of Temperature on Plankton Samples..... 15
Plankton in Relation to Bottom Texture..... 15
Supplemental Projects..... 16

UNIT IV - ARTHROPODS

Crabs..... 21
Barnacles..... 22
Supplemental Information..... 24

UNIT V - SALT MARSHES

Salt Marshes..... 34

UNIT VI - TIDAL POOLS

Tidal Pools..... 37

UNIT VII - POLLUTION

Introduction..... 39
Chemical Pollution..... 39
Microbiological Pollution..... 40
Test Procedures..... 40

UNIT VIII - MOLLUSKS

Population Study..... 49
Field Activities..... 50
Water Circulation in Mollusks..... 50
Mussel Dissection..... 51
Heart Rate of Clam..... 51
Burrowing of Clams..... 51
Supplemental Information..... 52

UNIT IX - MARINE WORMS

Field Activities..... 62
Oxygen Uptake of Clam Worm..... 62
Pulse Rate of Clam Worm..... 63
Volume Control in Estuarine Worms..... 64
Supplemental Information..... 65

UNIT X - NAVIGATION

The Magnetic Compass..... 68
Using a Marine Chart..... 70
Simple Course Plotting..... 71
Supplementary Piloting Problem..... 74
Field Activity..... 76

UNIT XI - PLANTS

A Study of Irish Moss..... 77
Supplemental Projects..... 78

UNIT XII - SPONGES & COELENTERATES

Sponge Structure..... 83
Sponge Regeneration..... 84
Supplemental Information..... 85

UNIT XIII - POLITICAL & SOCIAL INVESTIGATIONS

Political & Social Investigations..... 89

UNIT XIV - TIDES & CURRENTS

Current Bottles..... 97
Construction of a Simple Wave Machine..... 98
Current Velocities..... 98
The 24-Hour Clock..... 99
A Study of Tidal Ranges..... 100

UNIT XV - ECONOMIC INVESTIGATIONS

Economic Investigations..... 103

UNIT XVI - MARINE GEOLOGY

Bathymetric Analysis..... 106
Bottom Materials Analysis..... 106
Use of the Gravitational Core Sampler..... 107
Supplemental Projects..... 107

UNIT XVII - SPRING HERRING CENSUS

Herring Census..... 119

UNIT XVIII - ECHINOCERMS

A Study of Starfish..... 120
A Study of Sea Urchins..... 120
Supplemental Projects..... 121
Supplemental Information..... 128

UNIT XIX - SUPPLEMENTAL ACTIVITIES

Agar Digesters..... 133
Reference Tables & Graphs..... 136
Selected References..... 138

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INTRODUCTION

This lab manual was developed for use in an innovative program called Marine and Environmental Studies which was instituted in Warwick and Cranston in September, 1973. Funded under Title III of the Elementary and Secondary Education Act, the program is designed to capitalize on Rhode Island's greatest natural resource, Narragansett Bay.

Each school system is now equipped with a 19-foot Boston Whaler boat, trailer, and 12-passenger bus for use in this effort and thus has access to virtually every marine area in the state. During the winter months use of these boats is restricted but shore activities are still feasible and appropriate field trips can be conducted.

The program which is primarily field oriented offers the high school student full college and graduation credit. Approximately 63 students per community, in groups of seven, participate weekly in the program for three hours at a time. Thus the student must miss three hours of other classes. As such, the course represents an additional course above and beyond the student's normal load.

The organization of the units includes a section of supplementary activities which, while not covered during the normal class period due to time restrictions, may be beneficial to those students who wish to investigate a particular topic in more depth as a course project (required of every student). Quantities mentioned throughout the manual are for 7-8 students.

MARINE ENVIRONMENTAL STUDIES SYLLABUS

Fall

1. Familiarization with use of otter trawl; introduction to boating
2. Nekton
3. Fish tagging
4. Benthos
5. Plankton
6. Salt marsh
7. Rocky tide pools
8. Mollusks and marine worms
9. Pollution
10. Seining

Winter

11. Navigation
12. Tide and current study
13. Marine algae study
14. Political investigations
15. URI School of Oceanography
16. Pt. Judith Fisherman's Cooperative
17. Lafayette Trout Hatchery
18. RI Department of Health
19. RI Department of Natural Resources
20. New England Aquarium

Spring

21. Marine Geology
22. Barrier beaches; beach transect
23. Spring herring run study
24. Spring comparative trawling
25. Spring comparative pollution study
26. Student Projects

UNIT I

BEACHES

Background:

The growth of dunes along sandy seashores is a continuous and important geophysical part of the maritime environment. The dunes conserve fine sand moved off the beach by winds, they show a rapid, but narrow, elevational increase of the land, and they provide a first line of resistance to oceanic washover. The growth of dunes and the process of washover (being studied by other students) work together as part of the island building system. Washover deposits sand over a wide area as a result of a major storm, and it generally occurs infrequently. The source of this sand is both the beach and dunes. Once washover build-up occurs, dunes develop on the new surface and grow continuously as long as there is a source of sand and vegetation to trap the sand. Long intervals between major storms will result in substantial dune growth and the development of distinctive vegetation. The building of dunes is a continuous process and occurs whenever wind speeds are high enough to move sand off the bare beach. The sand stops moving, however, where it first meets resistance from beach vegetation. Here the dunes may grow a foot or more in a year. Wherever vegetation is lacking the sand is free to move. As dunes build, they provide protection for life in the interior of the island by reducing the amount of salt spray that comes across from the sea and by slowing down and reducing the frequency of washover. Such growth leads to relative stability so that other forms of life not adapted to salt spray and moving sand, as is the dune vegetation, can survive. In some areas, forest vegetation results from this stability and the forest will migrate in the direction of the most stable dune lines. Thus, plant succession follows the build-up of dunes. Many ecological lessons can be learned on the dunes, and it is impossible to cover them all in one exercise. Some famous ecologists have built their whole careers around the study of sand dune development and succession. However, we can start by making a few general observations on the growth of dunes and subsequent plant succession.

Keep in mind that we are observing an environment of constant change and flexibility. Nothing is stable for very long and a series of severe storms during a period of rising sea levels can cause drastic changes - dunes knocked down, forests destroyed, grasslands buried. Yet, in a short time, the islands recover, new dunes grow - and forests redevelop. This is how the barrier islands survive. Flexibility and change is the key to their continual existence in the face of the mighty sea. If sea level falls dune lines will follow the beach out as more land is exposed and the islands thus widen seaward. As sea level rises, dune lines are knocked back, washover pushes sand over the land, and the islands migrate slowly toward the mainland. You are to determine how your observations on dune growth fit into the studies being made by the other groups at work on the beach.

PROJECT: BEACH PROFILE

Objective:

To compare the profile of a beach from season to season, and explain any differences.

Materials:

String, stakes, meter stick, line level or protractor with plumbob, compass.

Procedure:

Establish several reference points in the dunes which will not change with the seasons and mark with stakes. Choose a point near the water's edge, set a stake, string a line, level, and take a compass reading from the reference stake. This same transect can now be set up at any time of year. Do this at each reference point. Starting from the reference point, using the meter stick, measure the distance from the level line to the ground, at a pre-determined interval, such as one meter. Using this data, construct a profile on graph paper. Carry out this procedure at three different times of the year: fall, winter, spring. Be sure to record date, and tide.

Question:

Compare seasonal transects by means of overlays or by plotting each season on the same graph, for each reference point. If there are any differences, explain.

PROJECT: BEACH TRANSECT

Objective:

To compare the zonation of life on a beach.

Materials:

1. clipboard - made of tri-wall with charts & directions
2. knife - putty type
3. plastic metric ruler
4. baggies & fasteners
5. thermometer
6. thread
7. paper towels
8. extra pencils
9. marking pencil
10. tide tables
11. small net
12. labels

Instructor - Should have some wrapping paper or poster paper, some string and markers. Plastic shoe box would be good if you wish to bring home some samples.

Procedure:

Record time, day, tide, date, weather conditions, etc. Give a brief description of the area. Always record any measurements taken.

I. Measurement of Surface Parameters

1. Temperature (shield bulb from direct sunlight)

a. 1 meter above ground
b. ground surface - on top of seaweed, if present
c. sub-soil or rock - under rock or seaweed
d. 15 cm below surface (if possible)
e. water temperature

2. Moisture (soil, sand or rock) - (dropsoil - could insert seaweed or surface covering)

a. dry - material falls apart
b. slightly moist - looks moist but doesn't stick together when squeezed
c. moist - sticks together when squeezed
d. very moist
e. wet - water drips out

3. Size of material making up substrate
Calculate by putting material on 9 cm^2 or palm of hand.

a. very fine - powdery
b. fine - can feel grains - impossible to count
c. coarse, 40-50 grams per 9 cm^2
d. very coarse 20 grams per 9 cm^2
e. stoney 5 pieces on palm of hand
f. rocky - stones larger than palm of hand
g. solid rock

II. Collection of Plant and Animal Materials

Pick: plant material - small pieces only of each type (species) that grows in or overhangs your plot. Do the same with each species of live animal. Put each sample in a separate baggie. Number same by placing in a bag or piece of paper with number on it. Identify if possible. MAKE SURE only a small piece/no. is collected. 'Conserve the natural area.'

1. Plant Life

Abundance - number of individuals of a plant type in the plot.

A = 10 individuals
B = 10 to 50 "
C = 50

If parts of plant in plot include
Cover - what percent of the plot does the plant type cover?
Estimate to nearest 10%

Density - degree of crowding - continuity

a. continuous
b. plants don't touch
c. scattered or in groves

2. Animal Life

Abundance - number of individuals of an animal type in the plot.

A = 10 individuals
B = 10-50 "
C = 50

Density - degree of crowding

a. continuous
b. do not touch
c. touch in some - scattered in others

Where Animals Live

a. in open
b. in shadows most of the time
c. under sheltering plants
d. under sheltering rocks
e. in sand or mud
f. in pools of water
g. on rock
h. additional - explore sand or mud, underneath rocks - use fingers, trowel and sieves. Return all rocks to their original places.

III. Debris

Dead material - Evidence of animal, plant life, including man. Put specimens in baggie - label.

Plant or animal debris - human debris.

p = plant
a = animal
h = human

Abundance

A = 10
B = 10-50
C = 50

Cover - To the nearest 10% what percentage of plot does debris cover?

Density

a. continuous - in piles
b. doesn't touch
c. scattered

Questions

1. Evidence of wildlife use - tracks, feathers, etc.?
2. Any potential wildlife food? (food chains)
3. Evidence of human activity.
4. Birds observed - habits and/or behavior.
5. Note adaptations of plants - of animals.
6. Power of erosion in the area.
7. Estimate how long area is without water, etc.
8. Locate plot as to zonation zone.

PROJECT: WAVE PROFILE

Objective:

To study characteristics of waves.

Materials:

Meter stick, stop watch

Procedure:

1. Wave Height:

   a. Move up and down the beach until your eye lines up with the top of a breaking wave and the horizon beyond. Mark the position on the beach.

   b. Measure the vertical distance (x) from your eye to the sand

   c. Calculate the vertical distance (y) from your feet to the lowest water level of swash as the water rushes back out to sea to join a new wave coming in. This would be the spot of exposed sand farthest toward the sea prior to a new swash coming in.

   d. Sight along a level stick to someone standing at this lowest point on the beach. Calculate y. $x + y$ = height of breaker.

   e. What is a spilling breaker? a plunging breaker?
   What is responsible for the height of breakers?
   What makes a wave break?

2. Wave Period:

Wave period is an important characteristic of waves. Period is the time it takes for two successive crests to pass a fixed point.

a. Observe waves passing a fixed point such as a pier or a jetty.

b. Count the number of waves passing the fixed point in 60 seconds.

Example: You count 8 waves in 60 seconds. What is the average period?

$$\frac{8}{60} = \frac{1}{x} \qquad : 8x = 60: \quad x = 7.5$$

Average period = 7.5 seconds

c. Does wave period vary from day to day, season to season, storm to calm weather?

UNIT II

NEKTON

Introduction:

Nekton is defined as any free-swimming organism, such as fish, whale, squid, seals, etc.

Fish Age Determination:

To the Fisheries Biologist the fish scale represents a valuable tool in the investigative process. He may choose to use the scale to determine the age of the fish. This information can be used in the interpretation of fish.

It should be mentioned, however, not all fish have scales, and those having scales do not all have the same type. Scales are not exclusive to fish. The more primitive forms have placoid scales. This is a scale possessing a dense enamel and dentine layers. Other fish scales are non-placoid types, having no enamel or dentine.

There are three types of non-placoid scales. First, the cosnoid type scale found in all lung fish of the world. The second type is the tile-like arranged ganoid scale found in gars and reed fish. Third type is the bony-ridge scale. Typically, they are thin and translucent. There are two types of bony-ridge scales. The first type is the ctenoid (teen-oid) having characteristically small sharp spines on the scale with no complete rings. The second type is the circular cycloid scale, a very easy scale for the beginner to work with.

When a fish emerges from the egg it is void of the characteristic scale covering of its parents. As growth of the fish begins, small scales appear just below the surface of the skin, the dermis. The scale continues to grow in proportion to the body. Thus, as the body grows a new ring of material is formed around the outside of each scale. This ring is called a circuli. The faster the body growth the greater the distance between the rings. Slower growth causes the rings to be closely spaced forming a dense-like band around the scale. This dense band is called an annulus. The slower growth rate normally occurs during the coldest months of the year. Thus in the warm season growth rings are further apart than in the winter months. Due to the stable environment in the tropics the annuli (plural of annuis) of tropical fish are less apparent or completely absent. The same would be true of aquarium fish.

Advantages of scales for determination of age in fish are very plain to the conservationist. First and most important you do not have to kill the specimen to remove a scale or two. Second, it is relatively fast, allowing the researcher the advantage of working on more specimens.

There are a few disadvantages also to the system. First it does not separate older aged fish easily. The older fish have a tendency to grow very little, if any in the later years, therefore, not producing annuli that can be easily recognized. Second, the hatching of a given species of fish does not always occur at the same time of year thus making comparisons more difficult.

It is said the most effective way to determine the age of a fish is through tagging, release, and recovery of the marked fish. The problems of this procedure are few but noteworthy. Fist is the cost of tagging alone. Second, the time involved in the entire procedure. Third, only a small number of speciments are usually recovered and returned, even if several thousand are tagged. Fourth, many specimens are injured from the excessive handling.

For the beginning student the scales to be viewed should be large and of the cycloid types. Scales should be removed from the specimen with forcepts from the area between the dorsal fin and lateral line. The collectors should place the scales in a glassine envelope. Field data required at the time of collection should include species, location collected, weight, length, sex if possible, method of capture and collector's name. This data should be kept with the scales at all times.

Fish Diets:

Unfortunately, it is difficult for most students to study the feeding habits of fish in the ocean, lakes and rivers. Since you cannot easily observe these organisms in their natural habitats, you will have to make inferences from the material available.

A certain plant population may be eaten regularly by one animal population which, in its turn, is eaten by another. These creatures may themselves be eaten by yet a third species of animals, and so on. Such a sequence is termed a food chain.

In the open sea the plants carrying on photosynthesis are free floating microscopic algae, principally diatoms and flagellates. Among the animals which eat these are tiny crustaceans known as copepods, important items in the diet of adult herrings (a bony fish). We write this simple food chain: Diatom copepod herring.

In nature the food and feeding relationships of plants and animals are rarely as simple as the example above. Copepods feed on a wide variety of species of both diatoms and flagellates. Similarly, the copepods, although they form some 21% of the adult herring's diet, are eaten by organisms other than herring; for example, they form 70% of the diet of larval sand eels, which themselves account for 40% of the adult herring's diet.

The simple food chain with which we started is not isolated, but connects with a large number of other food chains. This interconnection leads to the food relationships of organisms being thought of as a web. We refer to it as a FOOD WEB. In this case, the web might end in the herring being eaten by a gull - or you.

I. PROJECT: DIETS OF FISHES

Objective:

The purpose of this lab is for you to find out as much as possible about the eating habits and the processes of digestion in several species of fish.

Materials:

Student selected.

Procedure:

In order to study the eating habits of the organisms, you must know something about its anatomy, especially the digestive tract and its parts. Thus, you will be given a fish, and may use any methods you can think of to try to answer the following questions.

1. What is the scientific binomial (genus and species) of this fish? Common name?
2. What are the fundamental components of the anatomy of the digestive system of this fish?
3. What does the fish eat?
4. What happens to the food in the digestive tract?
5. What is the place of this fish in the food web of its particular environment?
6. How do pesticides disturb the food web? (Fish may retain pesticides for 4-12 months).

Supplementary Diet Exercise - Dissection

In order to understand the anatomy, be sure that you know what the following parts of the organism are, and their functions. Filmstrips and reference books of fish anatomy will help you to identify digestive organisms.

gills
gill rakers
pharynx
gall bladder
liver
stomach
sphincters
body cavity
doelom
"tube-within-a-tube"
pylorus
pyloric caecum
intestine
spleen
anus
esophagus
larynx
colon
mesentery

Try to lay out and trace the digestive tract from the mouth to the anus. (Do not cut into it yet.) Can you locate on your specimen all of the structures listed?

You may want to consider the following questions: Is there evidence from its external morphology that leads you to believe that the fish eats animals, plants, or organic debris? What are the maximum sizes of the material it must eat? Does it capture live food? Are you sure? Can you locate the gill rakers?

II. PROJECT: AGE DETERMINATION OF FISH

Objective:

To determine the age of fish by counting scale rings.

Equipment and Materials:

Fish scales or fish of various types and sizes
Hand lense, dissecting microscope, or compound microscope
Microscope slides and cover slips
Metric ruler
Drawing paper and pencil
Forceps
Paper envelopes

Procedures:

1. Remove a scale from the specimen with forceps from the area between the dorsal fin and lateral line.

2. Mount scale on microscope slide.
   a. Place scale on clean slide
   b. Carefully place cover slip on slide.

3. Determine scale type by use of a microscope or hand lens.

4. If the scale is a ctenoid remove another scale closer to the pectoral fin area and determine the type.

5. If cycloid type remove three additional scales.

6. Mount each as in item 2.

7. Observe scale, noting focus, annuli, circuli, radius if present, and ctenoid spine if present.

8. Determine age of fish by counting each annulus.

III. FISH TAGGING

Objective:

To study the aging, motility and/or migration of certain fish species.

Materials:

1. Depending on fish desired use one or more of the following:
   a. otter trawl
   b. rod and reel
   c. seines
   d. dip net

2. Spaghetti tags, fingerling tags, or Peterson disc tags.
3. Data card
4. Spring balance
5. Meter stick

Procedure:

After fish have been caught and before handling specimen wet hands to avoid removing protective slime from fish. Secure tag to fish just back of dorsal fin by using tag needle. Weigh and measure length of fish from mouth to fork in tail. Return fish to water as fast as possible to eliminate injury to fish. After fish has been returned to water record information on Data card. Information should include the following:

Date
Species
Sex, if possible
Weight
Length
Location caught
Method used
Age, if possible

Note to Instructor:

You should contact your local Fish & Game Division and work in cooperation with them.

SUPPLEMENTAL INFORMATION

Otter Trawl

The otter trawl is but one of many types of nets used for fishing and specimen collecting. Popular for research purposes is the quarter-scale nylon otter trawl, complete with doors, available from George Wilcox Co., Mystic, CT.

The otter trawl can be thought of as a large funnel made of netting which is towed in the water, large diameter end first. Heavy wooden doors are used to keep the sides, or wings, of the net open. A chain, or sweep, is used to keep the bottom of the net body (belly) down. Several floats affixed to the headrope keep the top of the net up in the water. Adding more such floats will cause the net to ride higher in the water. Thus the depth of the tow can be regulated. Line from the boat to the doors should be in proportion to the water depth at a ratio of 5 to 1. The fish and other organisms thus enter the net through the large opening and are caught in the narrow portion of the net, called the cod. Towing speed should be 2-4 knots.

UNIT III

MARINE PLANKTON

INTRODUCTION

1. Background

Of all the forms of life in the ocean, none is more abundant, diverse, and obscure than plankton: Organisms which drift, at the mercy of waves, tides and currents. Though the vast majority are microscopic, some forms are quite large, such as jellyfish and comb-jellies. The vast bulk of the ocean's life is planktonic and virtually all of the animals which live on the bottom or which swim freely feed on it. In fact, most non-planktonic animals have stages, usually juvenile, in the plankton.

It is of the utmost importance that we investigate this little understood but highly interesting group of sea dwellers - and that the conditions most favorable to their growth be perpetuated. For if we lose the plankton, we have lost all forms of marine life.

2. Collecting techniques

Plankton (drifters) may be collected in two ways: with a fine net or with a jar. It is far more desirable to use a net whenever possible, as the water will filter through it, leaving generally a far greater number of organisms caught in the net than one would collect by simply dipping a jar in the ocean. The tiniest organism (nannonplankton), however, cannot be collected even with the finest net made; thus the jar method is the only one available to us.

3. Gathering net plankton

There are two methods for gathering plankton with a net. Both presuppose moving water. The net, with a small bottle attached, is towed behind a boat. The boat must be moving 3-6 knots and the net should be towed between five and ten minutes. In doing comparative studies, decide upon a uniform towing distance for each sample. Care must be taken so as not to tangle the net or line in the motor or propeller. Or the net may be lowered into running water, such as under a bridge. It is desirable to determine the rate of flow of the water. This can be done by timing the flow of a stick over a measured distance.

When time is up, rinse out the net by raising and lowering into the water a few times. This washes the plankton into the bottle. If the bottle can be unscrewed from the adapter, do so. Otherwise reach down inside the net with your hand and pull the bottle up inside. Pour the contents into a small jar (such as a baby food jar) and be sure to add an equal volume of water from the same source. Why is this necessary? Label the jar as to

(1) date, (2) time, (3) place, (4) net size. Add any appropriate additional data. The lid should be tightly screwed on and the jar placed in a bucket of water to keep it as close to its natural temperature as possible until you are ready to work with it in the laboratory.

4. Net sizes

Several sized nets should be available. They are sized according to the mesh, the space between fibers, or the fineness. Here is a useful guide to net sizes:

Number	Aperture Size (mm.)	Used for
0	0.569	large zooplankters
6	0.238	larger zooplankters
12	0.119	zooplankton, large diatoms
20	0.076	most phytoplankton
25*	0.064	phytoplankton

*Nannonplankton (5-60 u) and ultraplankton (5 u) will readily pass through a #25 net.

5. Gathering nannonplankton

As previously noted, the only way available to us for collecting the tiniest drifters in the water column is to scoop them up in a jar. Back in the laboratory the organisms may be concentrated by spinning them down in a centrifuge. (A significant member of the nannonplankton in Narragansett Bay is the causative agent of our "red tides", a dino flagellate-like organism called *Olisthodiscus luteus*.)

I. PROJECT: COMPARING PLANKTON NET SIZES

Objective:

To compare the plankton samples collected with three or four sizes of net.

Materials:

Plankton nets, sizes 0, 6, 12, 20, and 25 (or any three or four of these); a transfer jar for each sample, a bucket, and labelling materials.

Procedure:

Tow each net as described previously. In order for a valid comparative evaluation to be made, what parameters must be constant? On-site comparisons may be made by simply holding up the jars side by side. Look for density, movement, organism size, and color. Then dilute and label each sample, placing them in a bucket of water to take back to the laboratory, or on the shore using a reflector microscope.

In the laboratory, if possible, examine each sample immediately with your

microscope, making sketches and indicating relative sizes of organisms. If it is necessary to keep the samples overnight or longer, add a drop or two of formaldehyde or FAA solution. This, however, will kill and decolorize.

II. PROJECT: VERTICAL TOWING

Objective:

To compare the results of vertical as opposed to horizontal sampling.

Materials:

#20 or #25 plankton net, weights (such as large fishing sinkers), transfer jars, labels, bucket.

Procedure:

Lower the net, with heavy sinker attached and boat stopped, to the bottom, then bring it up slowly. Do this several (perhaps ten) times. Determine the depth by measuring the length of line required. Then take a horizontal tow over a comparable range. Compare the two samples in the laboratory. (Project on page 16, Stratification of Plankton, should be done at the same time.)

III. PROJECT: PLANKTON IN A COVE AND IN OPEN BAY

Objective:

To investigate possible differences in quality and/or quantity of plankton in a cove and in the open water away from the shore.

Materials:

#6 or #25 plankton net, transfer jars, labels, bucket.

Procedure:

Select a zone in a cove, and tow for ten minutes so that the net is in the first half meter of water. Then using the same net, same depth, and same time period, tow the net offshore. In the laboratory, compare samples for kinds of organisms and density of life. How do you account for any differences observed?

SUPPLEMENTAL PROJECT: EFFECT OF A DELAY IN EXAMINING SAMPLES

Objective:

To test the effect of leaving unpreserved plankton samples overnight as compared to immediate laboratory analysis.

Materials:

#12 and either #20 or #25 plankton nets, 2 transfer jars for each sample, labelling materials, and a bucket.

Procedure:

Collect a plankton sample with each net you have. Divide each sample into two portions, labelling one TODAY and the other TOM'W. At the laboratory examine all TODAY samples carefully with microscopes. Place all TOM'W samples in the refrigerator until tomorrow. Examine them in the same manner the next day. Note similarities and differences.

SUPPLEMENTAL PROJECT: EFFECT OF TEMPERATURE ON PLANKTON SAMPLES

Objective:

To observe the effect of leaving a sample at room temperature or higher as compared to keeping it cool.

Materials needed:

Same as for experiment #2 above, except that you may want three or four jars for each tow.

Procedure:

Collect a plankton sample with each net you have, dividing it into three or four portions. Place one jar from each tow in a bucket of water. Leave the others at air temperature. In the lab, place the cool samples in the refrigerator, leave one sample from each tow at room temperature, and place one sample from each tow in a sunlit window. The next day, examine each sample comparatively, for both types and amounts of organisms. What can you conclude about the effect of temperature on plankton samples?

SUPPLEMENTAL PROJECT: PLANKTON IN RELATION TO BOTTOM TEXTURE

Objective:

To determine plankton differences drifting over rocky, muddy, and sandy ocean bottoms.

Materials:

#20 or #25 plankton nets, 3 transfer jars, bucket, labelling materials.

Procedure:

Make a tow over a rocky, a muddy, and a sandy bay bottom. Remember to tow for the same time and with the same net each time. Compare the contents in the laboratory. To what do you attribute differences in phytoplankton, if any? Do you find the different types of animal larval forms: If so, why?

SUPPLEMENTAL PROJECT: STRATIFICATION OF PLANKTON

Objective:

To observe the types of plankton found at different depths.

Materials:

#20 or #25 plankton nets, weights (several), labels, bucket.

Procedure:

Do this experiment in a location in the bay where the water is fairly deep. It is first necessary to determine, by geometry, at what depth the net is being towed using a given weight and moving at a given velocity. Take samples at various depths, from just below the surface to just above the bottom, perhaps at .5 meter intervals. Label jars as to depth. Examine by holding them up to the light on site, then microscopically in the laboratory. What evidence do you find of stratification? How can you explain it?

SUPPLEMENTAL PROJECT: MAKING A SINGLE SPECIES CULTURE

Objective:

To extract from a mixed plankton sample one or more members of a single species, whether phyto-or zooplankton, in an attempt to make and maintain a pure culture.

Materials:

Plankton sample (fresh), sterile culture vials, suitable pipette, sea water as a culture medium (artificial sea water).

Procedure:

Select a species you would like to culture by itself (perhaps a particular diatom, copepod, protozoan, water flea, etc.). From a slide of plankton from the sample, remove a single organism of your choice, using a suitable pipette (sterilized to prevent bacterial contamination). Place it in a sterile culture vial with sea water. (So that the sea water will be free from contamination, use artificial sea water, such as "Instant Ocean".) You may

find it necessary to bubble oxygen or to carefully maintain a certain temperature. This is a very difficult experiment, in terms of maintaining the culture you have made. Let it be a challenge to you.

SUPPLEMENTAL PROJECT: EFFECT OF CONCENTRATION OF THE SAMPLE

Objective:

To determine the effect of diluting a plankton sample on its vigor and longevity.

Materials:

Two or three fresh plankton samples, kept at the original temperature. Four transfer jars for each sample, additional sea water for diluting, graduated cylinder.

Procedure:

Divide each plankton sample into four equal portions (small), placing each in a transfer jar. Label the jars "1", "2", "3", and "4". Leave jar #1 as is. Add an equal volume of sea water to jar #2. Add twice as much sea water to jar #3. And 3 times as much water to jar #4. Store overnight under refrigeration. Examine each the next two or three days, both by holding up to the light, sniffing to detect decomposition, and microscopic examination. What conclusion can you make regarding the effect of diluting?

SUPPLEMENTAL PROJECT: ESTUARINE PLANKTON

Objective:

To collect and analyze plankton collected where fresh and salt water meet: the estuary; and to compare samples taken on incoming and on outgoing tides.

Materials:

#20 or #25 plankton net, transfer jars, labels.

Procedure:

From a bridge, drop your net into the water on an incoming tide. Pull it up after ten minutes, rinse and transfer the plankton sample to a jar, adding an equal volume of estuary water to dilute. Repeat on an outgoing tide the same day. In the laboratory, compare kinds of organisms present. Is the planktonic life in an estuary more or less diverse than in the bay itself? Why? How do the two samples taken differ?

SUPPLEMENTAL PROJECT: QUANTITATIVE ANALYSIS OF PLANKTON

Objective:

To discover a method used in sampling to determine the concentration of a plankton sample.

Materials:

Plankton sample, hemocytometer, microscope.

Procedure:

The enumeration of cells or other organisms may become a most routine and tedious task. It is, however, often the most important single index of experimental results. Magnification used is usually 100X with a 10X objective, although counting chambers designed for blood counts, is also ideally suited to small unicellular diatoms, although there is a tendency for it to discriminate against chain-formers. Each half of the chamber contains one etched grid (9sq. mm.) on a mirror smooth surface. With cover slip, each grid is calibrated to contain 0.9 ul. (microliter)of sample. A drop of the culture is introduced at the wedge-shaped opening to the grid at the edge of the cover slip. Rapid diffusion beneath the cover slip ensures an even distribution over the grid. If the distribution is not rapid and smooth, the slide should be cleaned and the procedure repeated. The chamber may be cleaned adequately by rinsing with fresh water, followed by alcohol, and wiping dry with cheesecloth. Usually 2 to 3 counts are sufficient to achieve acceptable accuracy. Be sure to convert to organisms per liter.

SUPPLEMENTAL PROJECT: SEASONAL CHANGES IN PLANKTON

Objective:

To detect, by regular collecting and analysis over an extended period, significant quantitative and qualitative changes in the plankton community in a restricted area.

Materials:

#20 or #25 plankton net, transfer jars, labels, bucket, preservative, and considerable perseverance!

Procedure:

Select an area to which you will have access throughout the time period during which you intend to pursue this project. Preferably once each week, on the same day, on either incoming or outgoing tide, take a sample. Keep all parameters constant throughout. You may wish to preserve, precipitate and weigh each sample to determine total biomass of plankton. Examine microscopically to determine dominant species. Make quantitative studies (see above, Quantitative Analysis of Plankton) of at least the phytoplankton. Chart and/or graph the trends you note.

SUPPLEMENTAL PROJECT: THE PLANKTON COMMUNITY AT NIGHT

Objective:

To compare and contrast nocturnal and diurnal plankton in a locality as to kinds, abundance, and levels in the water column.

Materials:

#20 or #25 net, transfer jars, labels, bucket, weights, lights.

Procedure:

Take plankton samples at various levels after dark (see page 16, Stratification of Plankton). Examine the samples on site without light (to detect bioluminescence) and with a flashlight. The following day, in the laboratory, examine microscopically, comparing with daytime samples you have previously examined. How does nocturnal plankton differ from diurnal samples? Why?

SUPPLEMENTAL PROJECT: PLANKTONIC COPEPODS

Objectives:

To learn (a) to identify copepods, (b) to identify their various life cycle stages, (c) to estimate the relative abundance of the various life cycle stages in a plankton sample, or a series of samples taken over a period of time.

Discussion:

WHAT IS A COPEPOD? A small crustacean, usually less than 6.5 mm. in length, visible in a plankton jar as being long and thin, with large antennae, and having jerking or darting movements. They are by far the most abundant (both in actual numbers and in biomass) herbivores in the sea, and, therefore, worthy of special study. Of the two pairs of antennae, it is the first pair ("antennules") which are elongated and prominent. The posterior tip of the abdomen is forked and with spines.

WHAT ARE THE PLANKTONIC TAXA? Altogether there are eight suborders of copepods, of which five are parasitic, and, therefore, not planktonic. The three planktonic suborders are as follows:

HARPACTICOIDEA: First antennae short (averaging 8 segments), body usually streamlined, with no obviously demarcation between metasome and urosome.

CYCLOPOIDEA: First antennae of medium length (averaging 16 segments), body divided into a metasome (broad anterior portion) and much narrower urosome (last thoracic segment plus forked abdomen).

CALANOIDEA: First antennae very long (averaging 24 segments, as long as the entire body), definite metasome and urosome usually present. This group is by far the most important in the open ocean.

WHAT ARE THE LIFE CYCLE STAGES? Among copepods, females bearing eggs can be distinguished in two ways: (1) the eggs may be borne in an external mass or two, attached to the abdomen, or (2) the female may carry a sperm case, a large oval pouch hooked onto her genital segment (the last thoracic segment). The juvenile stages are of the type called a nauplius, a segmented larva with, first, two anterior legs, then four legs (2 pairs), and eventually six legs (3 pairs), before molting and metamorphosing into a more adultlike stage called the copepodid. Egg-bearing females are most common in cold weather, with juvenile stages most numerous in spring and summer, copepodids being most numerous in fall.

Materials:

#12 plankton net, jars, labels, bucket. Diagram of life cycle stages of copepods and of the three planktonic taxa.

Procedure:

Tow the #12 net in the desired area for the usual period of time. (Using a #12 net will eliminate nearly all phytoplankton.) In the laboratory, scan a slide of the sample to determine (a) relative abundance of copepods, (b) which kinds are present, and (c) relative proportions of nauplius larvae and/or egg-bearing or spermatophore-carrying females.

UNIT IV

ARTHROPODS

PROJECT: CRABS

Objective:

To observe the behavior of a crab related to its structure and its environment.

Introduction:

The crab is an interesting example of a large group of animals called *crustaceans*, one of the classes of the arthropod phylum. You can learn a great deal about crustaceans, and something about animal behavior in general, by observing the structure and behavior of a crab. Refer to Page 24 for more material.

Materials:

Fiddler crab (or small crayfish, spider crab, rock crab, green crab, or blue crab), worms, snail meat, mealworms, or other food, probe.

Procedure:

Collect various types of crabs and proceed with following questions:

1. How does the crab walk? Poke it gently with probe if it does not move.

2. Describe the structure of the body. What evidence do you see of segmentation?

3. Pick the crab up by the middle, being careful to avoid the claws or *chelipeds*. Put it in the middle of the dry box or tank away from any stones. Where does the crab go? This reaction is called *positive thigmotropism*. What is its value?

4. Wave a pencil in front of the crab. Touch its claws and feelers. What does the crab do?

5. Put the probe in its cheliped. What does the crab do?

6. Put the crab in water. Touch its feelers with a probe. Describe the crab's behavior, compared to its behavior on land.

7. Does it have antennae (large feelers), or antennules (small feelers)? Does it have eyes? How are these organs built? Watch them for a few minutes. How are they used?

8. Feel the shell. Describe what you notice. Why is the term crustacean appropriate?

9. Feel some of the other crabs. If you notice anything different, explain your observations. What problem does the shell cause in relation to growth? How is the problem dealt with?

10. Describe the crab's color. How is this coloration useful?

11. Examine the appendages. Explain the name *arthropod* (jointed leg) for the phylum to which crustaceans belong.

12. If one of the appendages is broken off, it can regenerate (grow back). Examine your crab carefully. If you see any evidence of regeneration, describe what you found.

13. Feed the crab. How does it eat?

Conclusions and Discussion:

1. How is the crab's activities related to its body structure?

2. How does the crab's activities seem to be adapted to its environment?

PROJECT: BARNACLES

Objective:

To study the environmental adaptations of the Barnacles.

Introduction:

A barnacle is a crustacean, a relative of the crab, lobster, and sand flea, that has attached itself permanently to some object larger than itself. A shell has developed in which it lives, and its legs have become modified into efficient sweeps for the capture of small plankton and organic material on which it feeds.

There are four or five hundred species of barnacles in the world, many of which are highly specialized. For example one species is found only on the tongue of a certain turtle while another lives attached to the top of a whale's flipper.

If one examines a barnacle it is found to be composed of six triangular plates which form a conical shell. The opening at the top of the shell is closed by four movable plates which are hinged like folding doors. These plates serve a two-fold purpose for when the doors are closed the barnacle is protected from its enemies and from drying when exposed to the air. Inside the shell the body of the crustacean is enclosed in a membranous tissue or "mantle" which secretes the limy material of which the shell is made. The animal lies on its back with the cirri (specialized feet) pointing upward directly under the opening of the shell. The specialized feet or cirri are

composed of six pairs of legs, each of which has two branches made up of many segments. On the inner surface of each segment there occurs a double row of spines which forms a very efficient net for the capture of food. Sweeping motions of the feet direct food particles towards the mouth located near the middle of the body.

Growth is similar to other crustaceans in that at intervals throughout life molting or casting off of the old skin occurs. The new skin is flexible for a short while and growth of the body occurs at this time.

Econorically, barnacles prove to be a serious pest in marine circles as they encrust the bottom of ships and slow their movement. On the other side of the scale is the important fact that the young free-swimming larval barnacle are an important food source for different species of fish.

Reproduction of barnacles is hermaphroditic, that is, both male and female reproductive organs are present in each individual. The fertilized eggs are retained in sacs until they hatch into a free-swimming larvae. These drift at the mercy of the currents for a period of time and are increased in size by a series of "molts". The larval form eventually settles down and becomes attached at the head region. The adult shell is formed and the animal remains fixed in this position literally "standing on its head and kicking food into its mouth with its feet".

Procedure:

1. Examine the common beach barnacle and observe the four movable plates which form the "door". In order to observe a feeding barnacle, place a few in a tidal pool and wait quietly until the door opens and the cirri begin their rhythmic beating. The feathery appearance of tnese appendages are legs of the animal which have become modified for feeding.

2. Test the feeding barnacle for stimulus response by causing a shadow to fall across the animal or by touching the cirri. If the "door" closes the barnacle has exhibited a positive response to the stimulus. Further stimulus response testing can be accomplished by crushing a portion of a clam or a few barnacles and very carefully depositing a few drops (use a medicine dropper) of the resulting fluid near a feeding barnacle. Determine if this increases the action of the cirri.

3. Measure the diameter of one hundred or more barnacles using a pair of calipers and a metric rule. (Measure to the closest millimeter). Record and save the data for later classroom work. From this data you can determine the number of year classes (ages) of the barnacle found on the beach as well as the average yearly growth. (See chart in follow-up). Make sure that every barnacle in a given area is measured. (Measure small ones as well as large.) It should be noted that the more measurements are taken the more accurate the final analysis.

4. If a dissecting scope is available to use on the beach the barnacle could be examined microscopically. In order to do this the barnacle

must be removed from the shell. A pair of pliers will facilitate cracking of the shell and a probe and tweezers will aid in removal of the animal. If care is taken and sea water added to the dissecting dish, the animal may remain alive for some time. Examine the spines on the cirri and locate the mouth. Identify the digestive tract and look for eggs which may be attached to the body of the barnacle. Do not look for a heart for none is present. Body fluids flow or "slosh" around inside the animal by ordinary muscular movements.

5. The shell of the barnacle is composed of calcium carbonate. A common test for this substance is to add a few drops of dilute hydrochloric acid (HCI) or vinegar, on the object. If calcium carbonate is present, bubbles of carbon dioxide will form. Determine where the barnacle obtained the calcium carbonate. Make note of the fact that many marine animals use calcium carbonate in their shells. (i.e., crabs, clams, etc.)

6. Another problem is that of movement and spread of barnacles. First establish the fact that barnacles are incapable of movement from place to place as adults. Try to remove a barnacle from a rock. In doing so it will become evident that they are cemented tightly to the object upon which they are resting. Next determine how barnacles seemingly appear from nowhere on newly driven pilings and the hulls of new ships.

SUPPLEMENTAL INFORMATION

PHYLUM: ARTHROPODA

I. Phylum: Arthropoda (Arthropodes) (Jointed Legs)

A. Characteristics of the Phylum: Arthropoda:

1. Jointed appendages, which include legs and other body outgrowths.

2. A hard external skeleton or exoskeleton composed of chitin, (a hard substance.)

3. As the animal grows and becomes too big for its exoskeleton, it sheds it and secretes a new one. This process is called molting.

4. A segmented body; that is, a body with distinct divisions of the exoskeleton.

5. A dorsal heart; that is, a heart that is located above the digestive system. The circulatory system is of the open type.

6. A ventral nervous system, with the main nerves located below the digestive system.

7. Aerial, terrestrial, aquatic, and marine.

8. The largest of all phyla. Over three-quarters of all the animals in the world belong to this phyla.

II. Class insecta (This is the largest class. All insects belong to this class.)

A. Characteristics of the Class: Insecta:

1. The body is divided into three main regions:

a. Head
b. Thorax (chest region)

2. There are three pairs of legs present.

3. There are two pairs of wings if wings are present.

4. One pair of antenna.

5. The exoskeleton lacks lime.

B. In the study of marine biology, this class is omitted, since nearly all insects are either terrestrial or aquatic. There is one insect that is common in the marine environment; namely, Anurida maritima. The adult lays eggs which grow and develop in brackish water of tide pools in the superlittoral fringe.

III. Class Arachnida (Arachnids)

A. Characteristics of the Class: Arachnida

1. The head and thorax are usually fused to form a cephalothorax.

2. Antennae are lacking.

3. They have four pairs of legs.

B. Example 1: Limulus (horseshoe crab)(King crab)

1. Not a true crab.

2. Very common around our shores.

IV. Class: Crustacea (Crustaceans)

A. Characteristics of the Class: Crustacea:

1. Have two pairs of antennae (feelers) on the front of the body.

2. There are two distinct body regions as in the Arachnida:

a. Cephalothorax - includes the head and thorax (chest)
b. Abdomen - posterior region

3. More than five pairs of legs.

4. The exoskeleton contains lime.

5. Featherlike gills for respiration.

6. Mostly aquatic or marine. A few are terrestrial. Those that are terrestrial must live in moist places as under rocks.

B. Example 1: Crayfish - resemble lobster except that they are fresh water. Sometimes the word, crayfish, may be written crawfish.

C. Example 2: Lobsters

1. The common lobster that we enjoy in New England is Homarus americanus. This is the cold water lobster.

2. Spiny lobsters of the Florida and California coasts have no claws. They may grow over .6 meters long and weigh 13 kg. Most of them are less than 25 cm and weigh less than 0.9 kg. These lobsters have extremely long antennae and spines which cover the anterior region of the body.

3. General Information about the cold water lobster (Homarus americanus):

a. A 0.45 kg lobster is 5 years old. It has molted 20 times.
b. Adult female lobsters molt once every two years. They do not molt when they are carrying eggs externally.
c. Adult male lobsters molt every year.
d. Homarus americanus does not appear lower than the Carolinas.
e. Life cycle:

(1) Male places sperm which are stored in small sacs on the lower side of the female's body.

(2) From the time of copulation to egg release is about 9 months. At this time, eggs are fertilized.

(3) Female carries fertilized eggs on swimmerets. It takes about nine months for these to hatch.

(4) After hatching larva floats with plankton until after its fourth molt.

(5) After the fourth molt, they settle to the bottom and assume the shape of a lobster.

D. External Structure of the lobster

1. The body regions include the cephalothorax, which includes the head and thorax. It is covered by a hard plate or shell known as the carapace. The front part of the carapace that extends forward to form a protective beak is called the rostrum.

2. The abdomen, the second body region, is composed of 7 movable segments.

3. Compound eyes are located on either side of the rostrum. They are set on short movable stalks and are composed of numerous lenses.

4. Head apendages:

   a. Antennules(small antennae) - contain the hearing and equilibrium apparatus.

   b. Antennae - attached just behind the antennules. They function as organs of touch, taste, and smell.

   c. Mandibles (true jaws) These crush and chew food with the help of two pairs of maxillae or jaws.

      Note: The jaws work from side to side, not up and down. They are actually leglike appendages that are adapted for chewing, and they continue to move horizontally as legs do.

5. Thorax Appendages

   a. Three pairs of maxillipeds (jaw feet) - hold food for chewing.

   b. One pair of chelipeds (large claws) - sometimes called claw-feet. Their function is food-getting and protection.

   c. Two pairs of legs with tiny pincers at the tip.

   d. Two pairs of legs with a claw at the tip.

   e. Feather gills are attached to the maxillipeds, to the four pairs of walking legs, and to the chelipeds. These gills extend under the carapace to the gill chamber.

6. Abdominal appendages. (The abdomen is composed of 7 segments).

   a. Swimmerets are found on the first five segments. In the female, the last three pairs of swimmerets serve as a place of attachment for eggs.

   b. The sixth pair of abdominal appendages is much larger than the first five pairs. It is developed into a flipper or uropod.

   c. There is no appendage on the 7th segment. Instead this segment is reduced to a flat, triangular structure, the telson.

E. Digestive System

1. Food is held by the maxillae and maxillipeds while it is torn and crushed by the mandibles.

2. It then passes through a short esophagus to the stomach, which is lined with hard chitinous teeth. These grind the food into smaller particles. When the particles of the food are finely ground, they pass through folds of tissue, which act as a strainer, into another portion of the stomach where the food is mixed with digestive juices.

3. From here, the digested food passes into the digestive glands, where absorption takes place.

4. Undigested particles pass on through the intestine, instead of entering the digestive glands and are eliminated through the anus.

F. Excretory System

1. Liquid nitrogen wastes that result from the burning of foods in the individual cells are removed from the body by means of green glands, which lie anterior to the stomach and open at the base of the antennae.

G. Circulatory System

1. The blood of the lobster is pumped by the heart into seven large arteries that pour the blood over the major organs of the body.

2. Blood is collected in a number of sinuses or spaces. One large sinus surrounds the heart. From here, blood enters the heart through three openings called ostia.

3. As you can see, this is an open circulatory system.

4. The blood is colorless.

H. Nervous System

1. Similar to that of Annelids but is more specialized.

2. Receptors sensitive to odors and flavors are located in the antennae.

3. Each compound eye consists of numerous lenses, but sight is probably not keen.

4. The hearing apparatus is located in a sac in the basal segment of each antennule. Hearing is poorly developed.

5. Numerous sensory bristles that are sensitive to touch are distributed all over the body, especially on the surfaces of the antennae and other appendages.

6. The brain is dorsal to the digestive system on the anterior and of the body. A nerve collar leading from the brain surrounds a portion of the stomach and joins to form the ventral nerve cord. Numerous ganglia are found on the ventral nerve cord.

I. Respiratory System

1. Thin-walled gills, which are richly supplied with blood vessels, are well adapted for the exchange of oxygen and carbon dioxide between the lobster and its marine environment.

2. The gills are protected by a carapace, which extends over them and a chamber.

3. Water flows into the chamber under the free edge of the carapace, and is moved forward over the gills through the action of the second maxillae.

4. The gill chamber can hold enough moisture to permit the animal to remain alive for some time after it has been removed from the water.

J. Reproduction System

1. Sexes are separate.

2. Sperms leave the body through a duct that opens at the base of the fourth walking leg. In the female the opening for the egg is at base of the second walking leg.

K. Example 3: True crabs

1. The body of the crab is shorter and broader than that of the lobster.

2. Its abdomen is reduced in size and folded under the cephalothorax. This cavity formed in this way protects the gills and also serves as a brood pouch in which eggs are carried by the female.

3. Crabs are scavengers; that is, they will eat almost anything as long as it is dead.

4. Types of crabs

a. Blue crabs (Callicentes sepidus) are common in this area.

(1) Inhabit shallow bays
(2) If caught immediately after molting, they are called "soft shelled crabs" and are considered a delicacy. They can be cleaned, fried, and eaten without being shelled.

b. Hermit crabs (Pagurus longicarpus is common in this area)

(1) These are famous because they live in the empty shells of marine snails. As they grow larger, they must move to a larger shell.

(2) It is not certain whether they kill the snail or move in only if the snail is already dead.

c. Fiddler Crabs (Uca pugnax is common around here.) (Usually south of Boston.)

(1) One claw is larger than the other in the male. Males wave their large claw back and forth as though playing a violin.

(2) They are quite common in salt marshes.

d. Spider crabs (Sea Spiders) (Libinia emarginata is common in this area.)

(1) Have long spidery legs.

(2) When collecting them, use a separate container for each spider crab otherwise the legs tangle.

L. Example 4: Shrimp

1. Have 5 pairs of walking legs and a much enlarged and highly muscular abdomen.

2. They are stronger swimmers than crabs or lobsters. They swim backwards as do most crustacean.

3. In the Gulf Coast states and in California, the shrimp industry is very important. Louisiana, Texas, and California supply much of the shrimp to inland markets.

4. Maine shrimp (Shrimp from the waters of Maine) are sold during the month of February.

M. Example 5: Barnacles

1. Adults are sessile.

2. Free swimming larva (cypris larva) turns upside down and glues itself to the solid object by means of cements gland located in the anterior end.

3. Types of barnacles

a. Rock barnacles (Belanus balanoides is common in this area.)

(1) Possess a thick shell. They attach themselves to rocks and other stationary objects (i.e. piers, rocks, shells in

which hermit crabs live, to the body of animals like the whale, which often becomes irritated by them.)

(2) When the tide goes out, they close the 6 plates of their shells for protection. While under water, they thrust out their delicate fringed legs and kick minute organisms into their mouth.

b. Goose barnacles (Ship barnacles)

(1) They were considered by early zoologists to be mollusks, because they lived within a calcareous shell that they secrete.

(2) They possess a long stalk by which they are attached to seaweed or other floating objects.

4. Barnacles may accumulate on the hulls of ships in numbers large enough to reduce the speed of ships by as much as 20 per cent. They also clog seawater intake pipes and grow on piers. Here the accumulation of sand and decaying organic matter between the barnacle and the object to which it is attached provides a good medium where bacteria can grow and live.

5. Barnacles are usually associated with mussels. Together they change the environment of wharf pilings by providing protection for the living things that grow among them. The force of wave action is lessened, extreme temperature changes are prevented, and moisture is maintained among them, preventing drying at low tides. These environmental changes increase the rate of deterioration of the wharfs.

Note: The vast majority of Crustaceans occur as minute and even microscopic forms. The following examples illustrate this:

N. Example 6: Brine Shrimp (<u>Artemia</u>)

1. Inhabit tide pools and can survive in an environment of high salt concentration.

2. Many people who have tropical fish buy eggs of brine shrimp and raise the shrimp for fish food.

O. Example 7: Copepods (This is the name of a large group of small crustaceans.)

1. They are marine or aquatic. You have seen them swimming around in tidepools.

2. <u>Cyclops</u> (The water flea) is a very common copepod. It has one eye. They are so numerous that they color the water pink. They are freshwater forms.

P. Example 8: Isopods (This is a name of a large group of small crustaceans.)

1. They have seven pairs of identical legs. (This is what the name means.)

2. They are terrestrial, aquatic, or marine.

3. They lack gills but have a series of plates along the ventral surface of the abdomen with tiny tubes through which air can pass.

4. Since these plates must be kept moist for gaseous exchange (oxygen for carbon dioxide), terrestrial forms are found under stones and logs. (The sow bug and the pill bug are terrestrial isopods.) Terrestrial isopods can be kept alive for weeks in jars with potato or carrot slices available for food.

5. Idothea baltica is common in this area.

Q. Example 9: Ostracods (This is the name of a large group of small crustaceans.)

1. Tiny crustaceans that in addition to the exoskeleton, secrete two shells resembling those of clams.

2. They may be marine, aquatic, or terrestrial.

   a. Seed shrimp (marine)
   b. Daphnia (water flea) (aquatic)

R. Example 10: Amphipods (This is the name of a large group of small crustaceans.)

1. Aquatic or marine.

2. Range in size from 1 to 12 mm.

3. In this region, Gammarus locusta and Orchestia agillis (Eastern sand hopper)

S. The Development of Crustaceans

1. Let us take 5 crustaceans and list the different larval forms that they must pass through before they become adults.

Ostracods	Barnacles	Copepods	Lobsters	Crabs
egg	egg	egg	egg	egg
nauplius	nauplius	nauplius	nauplius	nauplius
adult	cypris	copepidid	protozea	portozoea
	adult	adult	zoea	zoea
			mysis	mysis
			adult	megalops
				adult

2. The following observations should be made:

   a. The more complex the crustacean, the more larval forms it passes through.

   b. The adult ostracod resembles a cypris larva.

   c. The adult barnacle resembles the protozoea larva.

   d. The crab is the most complex crustacean on this list. The last larval form that it goes through before becoming an adult (megalops) resembles a lobster.

   e. These larval forms are constituents of the zooplankton.

UNIT V

PROJECT: SALT MARSHES

Objective:

To study physical characteristics and biological organisms of the salt marsh.

Introduction:

Salt marshes are among the most productive areas in the world in terms of basic nutrients - proteins, carbohydrates, and fats. This may vary from producing 27.5 to 110 Kg of nutrients per acre per day.

Two and one-half tons of nutrients per acre per year are flushed into the sea as basic food for sea animals. These nutrients are passed along through a whole complex of food chains and webs and are essential to the production and maintenance of shell and fin, fish, waterfowl, and fur bearers.

Materials:

This list is rather large. It represents equipment that could be used for a variety of activities depending on the age and background of the group. The minimal equipment necessary is asterisked.

1. Camera (Polaroid, Instamatic, 35 mm, and close-up equipment.)
*2. Thermometers
*3. Hydrometer
4. Rulers and meter sticks
5. Clipboards and pencils
*6. Seine or crabnet
7. Corer
*8. Plankton net (refer to Page 13)
9. Shovel (Army surplus type), trowels and buckets or clam rake
*10. Sieve (½" and ¼" rabbit wire screen, a window screen, a kitchen sieve)
*11. Enamel pans (for sorting) or pails
12. Binoculars
13. Aquarium (to display speciments while in marsh)
*14. Collecting vials or baggies and rubber bands
*15. Formalin (4 - 6%)
16. Alcohol 60-70%
17. Press
18. Map of Area (could use map available at Refuge Center)
19. Crayons - water colors
*20. Sneakers or rubber boots
21. Insect repellant
22. Thread and string
23. Wooden floats

24. Hand lenses
25. pH paper
26. Forceps
27. Medicine dropper
*28. Identification Guide Books - Golden Series (Pond Life, Seashores)
29. Markers (3-8' bamboo poles)
30. Labels
31. Knife

Procedure:

Part I. When you arrive on side at the mouth of the creek, set up a series of markers (bamboo poles) about 30 meters apart along the side of the creek as it twists and turns its way toward the uplands (it may take a little time, but try to find a branch of the creek that reaches well inland). Number the points 1 - 3. Divide up your group and have them do the following:

1. Temperature reading of water - read at grass surface (edge of bank) and temperature about waist high when standing in grass.
2. Determining salinity - in all these cases it may be well to collect a bottle of water for these tests.
3. Determine velocity of water. Use wooden floats and a stop watch.
4. Take a pH reading.
5. Take a plankton sample - simply throw the net in and walk it against the current.
6. Describe the color of the water - hold sample against a white background.
7. Collect samples of fish, provided you have the proper equipment.
8. At each site collect samples of the plant life. Note size of plant, its abundance and its location with respect to the creek.
9. Take pictures of the area and close-ups of collected materials. Some students may want to sketch materials.
10. Carefully look around on the grass and exposed soil for signs of life. Ruffle up the grass, and examine these sites carefully.
11. Describe animals found such as snails; note where you found them. Are they in great abundance? Etc.
12. If you have a corer, take a core sample from the site. Describe soil texture.

Part II. Low Tide. Gather the group back at the mouth of the inlet. Proceed up the creek stopping at every stake and do the following (if equipment permits):

1. Observe and describe the bank profile. Dig into the bank and record its height from creek bed to upper surface.

2. Is there any life or evidence of it in the creek bank? Collect samples - record abundance and location.

3. Describe the bottom of the tidal creek. Feel and smell the sediment. What kind of sediment is it? Can you classify it as to sand, silt, or clay?

4. Is there any life or evidence of it on the surface of the creek? Collect samples - record abundance (A-abundant, C-common, O-occasional, R-rare) and location.

5. Take some surface material in a jar with some water - examine later for microscopic life. Describe what the animals are doing and what they are feeding on.

6. Do the same with the top 15 cm of sediment.
7. Are there any invertebrates in the sediment (dig in areas where you see holes). Use sieve and sorting pan.
8. Use camera as you did in Part I - get close-ups of life.
9. You may want to bring back some organisms for behavioral studies.

Part III. Tide on way in. Go back to the sites you were assigned to upon your arrival and repeat the following:

1. Temperature readings.
2. Salinity determination.
3. pH reading.
4. Plankton sample.
5. If netting available, try to capture some fish.
6. Describe color of water.
7. Velocity of the water.
8. Record any of the observations indicating differences between Part I and this part.

If there is any time left, you may want to run some tests and make some collections in the pans. Try a salinity reading.

Part IV. Follow-up in the field

1. Make a food web using the plants and animals you saw in the marsh.
2. How do organisms adapt themselves to life in a salt marsh - in brackish waters?
3. Did anyone see any evidences of pollution?
4. What protects the marsh from the sea?
5. What is so economically important (value of the marsh) about salt marshes.
6. What mammals make use of the marsh? Any evidence of their presence?
7. Using big paper sheets mounted on the side of a car or building, record data and group operations. Use one sheet for each testing point.
8. Discuss the tidal effect on the marsh. Are there times when the whole marsh is covered? How often? Were there any differences between the tests done as the tide was going out and when it was returning (Part I and Part III)?

UNIT VI

PROJECT: TIDAL POOLS - ROCKY SHORE

Objective:

To study the physical characteristics and biological organisms of a Tidal Pool.

Materials:

1. Camera
2. Thermometers
3. Hydrometers
4. Specimen Jars
5. Seine
6. Life Line
7. Sorting Pans
8. Guide Book (Seashores)
9. Fingernail Polish
10. Hand Lens
11. Microscope
12. General Preservatives
13. pH Paper

Procedure: I

1. Temperature readings; compare to temperature of open ocean.
2. Determine salinity.
3. Take pH reading.
4. Take plankton sample
5. Describe color of water
6. Take fish sample
7. Take a plant sample
8. Take picture of area including close-ups of collected material.
9. Take a population count of different animals present.

Procedure: II

1. Compare findings Procedure I to those of Salt Marsh, Unit V.
2. Note various differences. Could certain factors such as pH, salinity, and temperature change rapidly? Explain why.

Procedure: III (Lincoln Index)

The Lincoln Index, which is also known as the Peterson Estimate, is used to determine the population number of a specific animal population.

Marking Procedure

Collect a large number of a single species, dry and mark with bright paint or finger nail polish. Return samples to area of collection as soon as paint dries. Mark site of collection with a permanent stake. Record number marked.

Population Estimate

Complete the Lincoln Index within two weeks of tagging. Collect all marked and unmarked animals found within a 1.25 meter radius of the release stake.

1. Count the number marked
2. Count the number unmarked.

Do not collect marked samples found further than 1.25 meters from the release stake. Record an estimate of migration for each of these distant samples.

Estimate Population Size using Lincoln Index Formula

$$S_1 = \frac{(T)\,(C)}{(V)}$$

The Lincoln Index is based on the theory that the ratio of numbers tagged (T) to the population size (S), is the same as the ratio of tag returns (V) in a sample catch to the sample catch (C), i.e.:

$$\frac{\text{Number tagged (T)}}{\text{Population (S)}} = \frac{\text{Tag returns (V)}}{\text{Sample catch (C)}}$$

UNIT VII

POLLUTION

Objective:

To define pollution, both chemical and bacteriological, to learn how to measure pollution, to learn the effects of pollution, to learn current water standards, and to measure pollution in this state.

Introduction:

The amount of contaminants present in a body of water significantly affect the ecology and economics of an area. These contaminants, or those substances not naturally found in a body of water, are termed pollutants. It would be impossible within the scope of this course to study all the pollutants in an area; therefore several important contaminants and other variables have been selected and their significance is noted below.

In most cases the most efficient pollution study can be carried out using testing kits which are available commercially, such as Hach, LaMotte, and Millipore. At the end of this unit will be found several testing procedures which can be done from "scratch". Also, there are several water testing texts available, such as "Standard Methods for the Examination of Water and Waste Water" from the American Public Health Assn.

It is suggested that a field study include a comparison of "clean" and "polluted" areas, as well as comparison to standards set forth by the state and federal government. Rhode Island Department of Health standards are given in this unit, where such a standard exists. It should be noted that the ratio parts-per-million (ppm) is equivalent to milligrams per liter (mg/l).

CHEMICAL POLLUTION

Test	Significance	RI Standard - Class SA Water
Dissolved oxygen	Required for respiration; decreases as temperature increases	6.0 ppm or more
Ammonia	Blocks Kreb's cycle	none
Sulfide	Respiratory depressant	none
Heavy metals (Cd, Hg, Pb,etc.	Inactivates enzymes; act on nervous system, etc.	none
pH	Normal for ocean is about 8.0. If too high or too low, causes enzyme failure, tissue dehydration	6.8-8.5
Nitrate, Phosphate	Required by plants for protein, required by plants for nucleic acids, ATP, etc. When these are in large amounts, eutrophication occurs	none
Carbon dioxide	Required by plants for photsynthesis	none

MICROBIOLOGICAL POLLUTION

Bacteriological pollution is caused by contamination by human sewage. The principal problem organism is Salmonella typhosa, which causes typhoid fever. This organism is very difficult to test for, however, and normally Echerichia coli, as it turns out a harmless organism, is tested for instead. Thus the presence of coliforms presumes the presence of S. typhosa; the coliforms are termed "indicator organisms". Water contaminated with coliforms is bad, but eating shellfish which have lived in contaminated water is worse since, by their nature as filter feeders, they concentrate the organisms in their tissues.

Areas to be tested should be compared with the following abbreviated RI Department of Health standards:

TYPE OF WATER	Maximum Coliform per 100 ml
SA (suitable for all uses)	70
SB (shellfishing prohibited)	700
SC (bathing and shellfishing prohibited)	Anything over 700

Procedures (if kits are not used):

I. Dissolved Oxygen Concentration

Generally, aquatic organisms require concentrations of oxygen of not less than 5 mg per liter or 5 ppm. If water contains less than this amount, a number of biological processes may be interferred with. Some of the processes which may be interferred with include swimming performance, embryo size, and growth.

Whether or not excess oxygen poses a problem for aquatic organisms, has yet to be determined.

Ordinarily, oxygen is not a limiting factor in the oceans because of the effective mixing processes which occur. However, in a bay area, dissolved oxygen should be determined.

We must also consider that: (1) as the temperature increases, oxygen consumption by aquatic organisms increases; (2) in some aquatic organisms, as the oxygen content is lowered, the respiration rate increases; (3) some aquatic organisms will acclimate to lowered oxygen concentration whereas others will not.

Bearing in mind all these considerations we use what is known as the Rideal-Stewart modified Winkler test to determine the dissolved oxygen concentration.

You will first want to secure water samples from each of the two areas you are going to study. Glass bottles with tight-fitting glass stoppers and having a capacity of 250 ml are used. Glassware should be clean.

If special water samplers are not available, avoid splashing or bubbling the water. Siphons may be of use or the water may be taken directly into the bottle at a 45° angle with a gentle stream of water to avoid turbulence or air bubbles. If a sampler is available, water can be easily secured without introducing atmospheric oxygen. If water is then transferred from the sampler to the bottle, the water should be allowed to overflow the 250 ml bottle 2-3 times to flush out atmospheric oxygen. When the stopper is replaced, no air bubble should remain. The temperature of the water should be recorded at the time and place the water is collected (see procedure on following pages for determining temperature).

Reagents

All water used is distilled.
Potassium permanganate solution: 6.32 g KMnO in 1 liter H_2O.
Potassium oxalate solution: 20 g $K_2C_2O_4 \cdot H_2O$ dissolved in water. Add 4 g NaOH and dilute to 1 liter with water.
Manganous sulfate solution: 480 g $MnSO_4 \cdot 4 H_2O$ to 1 liter of water.
Hydroxide - sodium iodide solution: 500 g NaOH and 135 g NaI to 1 liter of water.
Sodium thiosulfate: (N/10): 24.82 g $NaS_2SO_3 \cdot 5 H_2O$ to 1 liter of water. Use cooled boiled water. Add 5 ml of chloroform. When this is to be used, dilute to N/100 by adding 9 parts water to one part of this solution.
Starch solution: 3 g potato starch, ground with H_2O. Place in 500 ml freshly boiled water. Allow to stand overnight, then use only the clear fluid.

For the following steps (1-10) you should add the reagents quickly and restopper the bottle to prevent oxygenation from the atmosphere. Steps 1-6 must be carried out in the field.

1. To the water sample add:

   a glass bead to aid in mixing
   .7 ml concentrated H_2SO4
   1.0 ml $KMnO_4$ solution

2. Shake well. A pale violet to pink color should appear and persist. If it does not, add another ml of $KMnO_4$ solution. After the color is established, allow the sample to stand for at least 40 minutes.

3. Add 1.0 ml potassium oxalate solution. Let stand until the color disappears.

4. Add: 1.0 ml manganous sulfate solution
   3.0 hydroxide sodium iodide solution

5. Shake. A yellow precipitate will form. Allow this to partially settle and then shake again.

6. Add .5 ml of concentrated H_2SO_4. The precipitate should dissolve. If it does not, add another .5 ml of the acid. The yellowish color remaining represents the iodine which has replaced the dissolved oxygen. At this point analysis may be suspended for some time, allowing the titration to be carried out in the laboratory.

7. Measure out 100 ml of the water sample and titrate with the sodium thiosulfate solution. MAKE SURE THAT YOU READ THE LEVEL OF THE SODIUM THIOSULFATE SOLUTION BEFORE YOU START TITRATING. WRITE IT DOWN. Titrate until a very pale yellow color is reached.

8. Add 2 ml of starch solution. The sample will turn blue.

9. Continue titrating until the sample becomes clear. The clearness should persist under agitation.

10. Calculate the amount of sodium thiosulfate used, in ml, and multiply by 4/5. This will give roughly the parts per million of dissolved oxygen in the sample of water. More exactly, PPM dissolved oxygen =

$$\frac{800 \times \text{ml thiosulfate used} \times \text{normality of thiosulfate}}{\text{ml of sample titrated}}$$

II. Free CO_2 Concentration

Carbon dioxide concentration is important for several reasons. From an economic viewpoint, this gas contributes to several forms of corrosion. From the biological viewpoint, less than 25 PPM of carbon dioxide can be lethal to aquatic animals. In addition, a high carbon dioxide concentration usually means a low dissolved oxygen concentration.

An increase in the carbon dioxide concentration usually results in avoidance reaction by fishes.

Obtain a water sample and with it fill a Nessler tube to the 100 ml mark. Be careful not to splash or agitate the water, since carbon dioxide will easily come out of solution. Proceed immediately:

1. Put in 10 drops of phenolthalein solution. (5 g phenolthalein in 1 liter of 50% alcohol, neutralized with N/50 NaOH).

2. Titrate with N/44 NaOH. Be sure to write down the level of NaOH before starting the titration.

3. The end point is reached when a pink color appears for a few seconds under agitation. DO NOT titrate until a permanent color forms.

4. Read the burette after titration, calculate the amount of NaOH used.

5. Multiply this amount by 10. This will give the amount of free CO_2 in PPM that was in the sample.

III. Temperature

All aquatic animals are poikilothermic. By this we mean that they depend upon their environment for their body temperature. Generally, as temperature increases, activity increases as does the oxygen consumption.

Aquatic animals can become acclimated to higher or lower temperatures to some extent.

Elevated temperatures can: (1) increase oxygen demand; (2) decrease the organisms' capacity to use dissolved oxygen; (3) decrease the solubility of oxygen in the water and even (4) increase the toxic effects of poisons.

Since air temperature determines pretty much the surface water temperature, it is important to know. You may use an ordinary thermometer to take the air temperature but you must make your reading in the shade (place your hand between the bulb and the sun).

It is usually best to take a temperature series. A reading should be taken at every 1.5 meters interval preferably using a small, battery-powered thermistor. A maximum-minimum thermometer may also be used, resetting the indicator markers each time to record the temperatures. The least accurate method is to use a collecting bottle to obtain a water sample from the desired depth. Quickly raise it and take the temperature before it has a chance to change too much.

IV. Hydrogen Ion Concentration

In general, waters having a pH value of from 6.7 to 8.6 are favorable to aquatic organisms. Fishes are usually tolerant of hydrogen ion concentration even slightly beyond this range.

You can obtain the approximate value of hydrogen ion concentration by using special indicator papers. These are merely dipped in the water, and the resultant color is compared to a color standard. This type of test is not precise, and gives only an indication of the pH of the water.

V. Total Suspended Solids

Large amounts of suspended material in water can be harmful to aquatic organisms. The suspended material will obstruct light inhibiting photosynthesis and reducing biological productivity of an area. It can bring about an increase in the temperature of the water. It affects the feeding process of mollusks. Gills of fishes may not function at maximum efficiency.

One cannot anser the question of how much material may be harmful to aquatic organisms. Oysters, for example, are very sensitive. Eggs in concentrations of 1 or 2 g per liter of silt have a survival rate of near 0.

For our study we shall consider large amounts of suspended materials to be in the range of 90 to 810 PPM. According to the U.S. Public Health Service, water for human consumption should have a turbidity of not more than 10 PPM.

Generally,the following criteria have been established which may be applicable here:

25 - 30 PPM	Optional
30 - 85 PPM	Good
85 -400 PPM	Poor
400 and up	Extremely bad

Although these figures were established for stream quality, they may serve as useful guidelines.

1. Weigh a piece of fine filter paper.

2. Filter a 1 liter sample of water through the fine filter paper.

3. Allow the filter paper to dry completely.

4. Reweigh the filter paper. The change in weight is the weight of the total suspended solids in 1 liter of water. T.S.S. values are commonly expressed in PPM (mg per liter).

VI. Transparancy

Another method of indicating suspended matter in the water is by using a Secchi disc. This can easily be made. Obtain a metal disc, 20 cm in diameter and divided into four quarters, 2 of which are white and 2 black. With this instrument you can obtain a rough measure of the amount of suspended material; the depth of the reflected light penetration, and an estimate of the extent of the littoral zone. It will be necessary to attach a cord of some sort to the center of the disc so that it can be lowered into the water.

Lower the disc into the water until it just disappears. This must be done in the shade. Record the depth of the disc. Now raise the disc until it just reappears. Record the depth at this point. Average these two measurements. Repeat the process three times and the overall average is considered to be correct. Note the surface conditions and the color of the water. You can obtain much better measurements if you use a glass-bottomed bucket to look through since this will eliminate surface distortions.

A low average (e.g. 1.5 meters) indicates that the water contains much suspended material. A high average (e.g. 9 meters) indicates that the water is free of suspended material and clear.

The average also corresponds to the depth limit of the littoral zone, i.e. where you would find rooted plant growth.

VII. Hardness

The hardness of the water usually refers to the presence of certain metals (cations of) and the elements that combine with them to form salts. Generally, hardness is the result of the presence of the cations of

Calcium and Magnesium, however large amounts of minerals such as Potassium, Sodium, Silicon, may also be indicators. There are certain tolerance limits of marine organisms to metals and their salts. Extreme hardness usually is toxic to many marine organisms. Our investigation will give us the hardness in terms of calcium carbonate in PPM. It might be of interest to note that several species of marine fishes can only tolerate 2.4 gm/liter of calcium chloride in sea water, 1.5 gm/liter of Magnesium chloride and 0.1 gm/liter of Potassium chloride. With this information, one can get a picture of some of the toxicity levels to certain metals as chloride salts and perhaps make some conclusions about the effect of hardness readings which they obtain of the marine organisms.

The soap method for determining hardness will probably have an acceptable error of ± 10 PPM. *Prepare materials 1-3 days in advance.

1. Place a 50 ml water sample into a 250 ml bottle.

2. Add a standard soap solution - shake 100 gm of pure castile soap powder into 1 liter of 80% ethyl alcohol. Cover and let stand for 1 to 3 days, then decant the upper layer. Discard the soap left in the bottom of the bottle. Dilute a small portion of this solution with 80% ethyl alcohol until 1 ml is equivalent in hardness to 1 ml of standard calcium chloride solution (see below).

   To make the standard calcium chloride solution:

   Dissolve 0.5 gm of anhydrous calcium chloride in a few ml of dilute hydrochloric acid. Add 200 ml of carbon dioxide - free distilled water. Neutralize the solution with ammonium hydroxide until it is just alkaline (use litmus as an indicator). Add further distilled water until the volume of the solution is 500 ml. Store in a glass container. One ml of this solution is equivalent in hardness units to 1 ml of standard soap solution. Thus it is necessary for you to test the soap solution and adjust its concentration until 1 ml of the solution just forms a permanent froth when it is shaken with 1 ml of the standard calcium chloride solution.)

3. When a lather is first seen, let the bottle stand for 5 minutes. If the lather remains, the end point is reached. If the lather disappears, continue to add soap solution until the lather does remain.

4. The number of ml of soap solution used, multiplied by 20, gives the hardness (in terms of calcium carbonate) in PPM.

VIII. Alkalinity

Probably the greatest cause of alkalinity in water in this area is pollution from laundries. There are many other sources such as tin plate and wire mills, starch factories, cloth sizing mills, to name only a few. All of these may increase the alkalinity to the critical point. The carbonates, bicarbonates and hydroxides of calcium, magnesium, sodium and other metals result in what we call alkalinity. We will express alkalinities in PPM as an equivalent amount of Calcium carbonate. *Titrate over a white surface so that color changes can readily be seen.

1. To a 100 ml sample of the water to be tested add 5 drops phenolthalein indicator solution.

2. If there is no change in color go right on to step 4 (omit 3). If there is a color change, go to step 3.

3. Titrate with 0.01 M sulfuric acid until the color disappears. Record the number of ml used in the titration. Multiply the number of ml of acid used by 10. This is a measure of the phenolthalein alkalinity in PPM as Calcium carbonate. (This measures the amount of hydroxide and half the amount of carbonate in the water). Go on to step 4.

4. Add 5 drops of methyl orange to the solution from step 2 or 3. If the solution becomes yellow, titrate with the 0.01 M sulfuric acid until a pinkish color appears and persists. Record the ml of acid used.

5. Total alkalinity is the sum of the ml of acid required for both titrations, multiplied by 10. This is the total alkalinity in PPM as Calcium Carbonate.

IX. Ammonia

Ammonia is one of the poisons in the sewage which is important with respect to the killing of fishes. It is also a waste material from industrial chemical operations, ice making plants, and large cleaning operations involving the use of large amounts of ammonia water. Water with less than 0.2 mg per liter of ammonia as nitrogen is considered clean.

In considering the toxic effect of ammonia on organisms we must first note that toxicity depends directly on the pH of the water. For example, water with significant amounts of ammonia and ammonium salts but a low pH value may not be toxic. Other factors which influence the toxicity of the ammonia are the presence of carbon dioxide and the concentration of dissolved oxygen. Carbon dioxide reduces the toxicity of the ammonia. When the concentration of dissolved oxygen is low, the toxicity of the ammonia is increased. Other factors include bicarbonate alkalinity as well as temperature.

We may consider the following in determining a safe level of ammonia in water for fishes. Concentrations of 2.5 mg of ammonia per liter are generally harmful in the pH range from 7.4 to 8.5. Other studies reveal that concentrations of 1.5 mg of ammonia per liter are harmful to many fishes.

The method we will use to determine the ammonia as nitrogen is the A.P.H.A. Nesslerization Method.

1. Obtain a 25 ml sample of the water to be tested.
   Add 1 drop of Rochelle salt solution to sample.

2. Add 20 drops of Nessler's reagent to the sample and mix gently by swirling.

3. A yellow color indicates that ammonia nitrogen is present. It will take at least 10 minutes for full color to be achieved.

X. Coliform Bacilli

We will consider that the presence of any kind of fecal coliforms indicates that the water is polluted. Coliforms are usually found in the intestines of man and animals. These organisms are defined as short, gram-negative rods that ferment lactose forming acid and gas. They are facultative anaerobes and multiply rapidly between 30 and 37°C.

Before beginning your tests, familiarize yourself with correct microbiological techniques. Prepare all tubes and plates in advance.

Presumptive test

This is based upon the ability of coliforms to ferment lactose and produce a gas. Since other organisms can also ferment lactose, we can only presume that coliforms are present. Nutrient broth with 1% lactose will suffice. You will use Durham fermentation tubes for this test (test tubes with small inverted vials for collecting the gas).

Confirmed Test

The presence of coliform bacteria is indicated by the formation of typical colonies on E.M.B. agar. Colonies of E. coli are 2 to 4 mm. in diameter, have a large, dark or even black center, and have a green, metallic sheen when observed by reflected light. Colonies of A. aerogenes are larger, mucoid and pinkish; they often have a brownish center.

1. Streak E.M.B. agar PLATES from several of the fermentation tubes containing gas. Incubate at 37°C. for 18 to 24 hours.

2. Typical coliform colonies indicate a positive confirmed test.

3. No colonies indicate a negative confirmed test.

(If colonies are present and are not typical, it is advisable to continue with the completed test.)

Completed Test

Inoculate lactose fermentation tubes and nutrient agar slants from the typical or atypical colonies obtained above. Incubate at 37°C for 24 hours. If no gas appears this indicates a negative completed test, i.e., coliforms are absent. If gas appears:

1. Prepare smears on slides using heat for fixing. (You can either place the slide over an incandescent light source or briskly move slide through a bunsen burner flame.)

2. Add 1 drop of crystal violet and allow to remain for 1 minute.

3. Rinse with tap water (not more than 2 sec.).

4. Add 1 drop of iodine solution for 1 minute.

5. Rinse gently with tap water.

6. Carefully blot dry. Rinse gently with 95% ethanol for 30 seconds.

7. Blot dry again. Add 1 drop of safranin for 1 minute.

8. Rinse with tap water, air dry and examine.

Observe under the microscope. Gram-positive bacteria appear dark violet. Gram-negative ones appear red. If your smear is gram-negative with no spores then you have a positive completed test indicating that coliforms are present.

UNIT VIII

MOLLUSKS

Objective:

To study various adaptations of certain mollusks.

Field Activities:

I. Population Study

Purpose:

In this activity we will undertake a population study of the common blue mussel in order to determine if different habitats affect the size to which blue mussels grow.

Procedure:

Collect samples from two different mussel populations. One area should be an exposed rocky area, the other a protected habitat such as a mud flat or dock piling. Examine the samples and record their general characteristics.

To what broad animal classification do they belong? (phylum)?

What purpose is served by the thread-like structures which you will note on many of the specimens?

Data:

Choose a size range of 4 or 5 increments which will be suitable for size grading each of the samples, (e.g.) 1.0, 2.0, 3.0, 4.0 cm. Measure length of mussel.

Record increment totals below

Sample One		Sample Two	
Location_____		Location_____	
Size	Number	Size	Number
____	_____	____	_____
____	_____	____	_____
____	_____	____	_____

Conclusion:

Is there a difference in the size range of the two samples? If so, how might we account for this?

II. Other Field Activities

1. Collection

2. Identification

3. Classification

4. Importance and biological peculiarities of certain species

5. Aging techniques

6. Good Rhode Island locales:

   a. Narrow River - oysters

   b. Sandy Point (Potowomut)

      Whelks
      Blue mussel
      Ribbed mussel
      Ark shell
      Slipper shell
      Angel wings
      Soft shell clam
      Hard shell clam
      Scallop
      Jingle shell
      Periwinkles
      Drills
      Dog whelks
      Razor clam

Classroom Activities

I. Water Circulation of Mollusks

Purpose:

Illustrate the method of food intake and water circulation in Mollusks.

Procedure:

1. In a small quantity of sea water add a few drops of vegetable dye. Let this solution acclimatize to room temperature before using.

2. Draw 5 ml of dye solution with pipette, release near opened shell or siphonal canal of bivalve.

3. Observe intake and release dye.

4. Compare species:

   a. By presence or absence of siphonal canals.
   b. Direction of dye intake and release.

II. Mussel Dissection

Purpose:

Undertake a gross examination of the internal anatomy of the common blue mussel.

Procedure:

You will work with moderately large, living specimen taken from a habitat where they are found in abundance.

1. Describe its exterior appearance. (sketch)

2. Using a scalpel carefully open your specimen: Note the "soft body".
   What Phylum does it belong to?
   Locate the much reduced foot.
   Locate the mantle. What is its function?
   Find the gills. What is their purpose?

3. Carefully remove a small portion of a gill. Place it on a slide and examine under the microscope using various magnifications. Describe what you see happening.

Conclusions:

What purpose do you think is served by the action you have described above?

What would you guess makes up the food supply of this organism?

III. "Heart Beat"

Materials:

1. Insert the point of the knife between the valves near the hinge and very carefully cut around the top of the pallial muscle keeping the knife pressed against the inner surface of the shell and cutting both adductor muscles.

2. Separate the top shell from the mantle and body portion of the clam.

Observe:

1. The slow and irregular beating of the heart near the hinge of the clam.

IV. Burrowing Activities of Soft Shell Clam

Materials:

One pan or dish containing several inches of sand or other sediments, several clams (small clams will be better actors), fresh seawater, (or carry on the experiment out on the clam flats in a shallow pool of water).

Procedure:

Place the clams on the surface of the sediments.

Observe:

1. Action of the foot in being extended from between the shells.
2. The attempts and possible success of "anchoring" by the foot in the sediments.
3. The lifting of the posterior or siphon end of the shell to an upright position.
4. The burrowing action with the opening and closing of the shell by the use of the adductor muscles.
5. The surge or flow of water up around the clam as water is jetted from the pedal opening and the clam digs into the sediments.

Conclusion:

Small clams burrow actively, particularly in the warmer months.

Note: The clams may not always cooperate immediately and several attempts may be necessary before the observations can be made.

SUPPLEMENTAL INFORMATION

PHYLUM: MOLLUSCA (MOLLUSKS)

I. Phylum: Mollusca (Mollusks)

A. Characteristics of the Phylum: Mollusca:

1. Many possess shells of calcium carbonate.
2. Many possess a foot.
   a. The snail uses it for creeping.
   b. The clam for plowing in the mud.
   c. The squid for seizing prey.
3. The internal organs are covered by a layer of tissue, called the mantle. It is the mantle that secretes the shell. The space between the main body and the mantle is called the mantle cavity.
4. The anus opens into the mantle cavity.
5. They eat both plant and animal food.

6. Respiration takes place in gills, or mantle, or both.

7. Sexes are usually separate; some are hermaphroditic.

8. They may be terrestrial, aquatic, or marine.

9. The terrestrial mollusks and most marine forms pass through the trochopore larval stage while confined to the egg capsule.

Note: Annelids also go through a trochopore larval stage. As a result, biologists consider them to be related to Mollusks.

II. Class: Gastropoda(Stomach arch footed) (Univalves)

A. Characteristics of the Class: Gastropoda

1. Flat-footed, with or without a coiled shell.

2. Head, distinct eyes, and tentacles are present.

3. Has a one-piece shell.

B. Example 1: Snails (terrestrial, aquatic, and marine)

1. Oxygen diffuses through the mantle. Since this must be kept moist, the snail must not dry out. To prevent drying out during dry seasons, snails retire into their shells as fast as possible and secrete a parchmentlike wall across the opening. This prevents evaporation.

2. Snails move as follows:

a. A slime gland at the forward end of the foot deposits a film of mucus on which the snail moves by means of wavelike contractions of foot muscles. Progress is always about the same. (Approximately, 5 cm. per minute.)

3. Most snails are 'right handed' (dextral). Here's how to tell:

a. Hold the shell in front of your eye with the foot toward you.

b. If the opening is on your right, it is right-handed (dextral). If the opening is on your left, it is left-handed (sinistral).

4. When snails open their mouth a tonguelike structure comes out of the mouth and scrapes the glass of the aquarium. This is the radula (scraper). It is like a file and scrapes algae from the glass side of the aquarium. You can identify a species of snail by its radula.

5. Many snails serve as intermediate hosts for flukes.

C. Example 2: Periwinkles (usually smaller than snails). The common periwinkle that we find around here belongs to the genus: Littorina.

D. Example 3: Slugs (terrestrial), nudibranchs (marine). It resembles a snail that has lost its shell. Garden slugs along with garden snails can cause damage to gardens as they feed on leaves.

E. Example 4: Whelks.

F. Example 5: Abalones. (Fried abalone is a famous dish on the west coast.)

G. Example 6: Conches (Found in semi-tropics or tropics).

H. Example 7: Cowries (Found in tropics or semi-tropics).

I. Example 8: Limpets (Grow attached to rocks).

J. Example 9: Crepidula (Slipper shelled limpet) - oldest member is female while younger members of the cluster are males. (They contain both male and female organs.)

K. Example 10: Cone shells (Found in tropics or semi-tropics).

Note: Pictures of most of these mollusks can be found in the Golden Nature Guide, Sea Shells of the World.

III. Class: Pelecypoda ("hatchet-footed", two-shelled mollusks) (bivalves).

A. Characteristics of the Class: Pelecypoda:

1. Axe-footed with bivalve shells. (Two shells connected by a hinge.)

2. Head, eyes, and tentacles lacking.

B. Example 1: Clam

1. Some are freshwater (aquatic); most are marine.

2. Types of clams:

a. Mya arenaria - soft shelled long neck clams. This is the type that we use when we fry clams or have steamers.

b. Venus mercenaria - hard-shelled littleneck clam or quahogs. Cherrystones are young quahogs.

c. Anedonta - a common freshwater clam.

d. Spissula (Mactra) solidissima (surf clam).

e. Fnsis directus - razor shell clam.

f. Tridacna gigas (Giant Clam) - May reach a length of 1.5-1.9 meters and weigh 225 kg.

3. The shells are held together by muscles called the anterior and posterior adductor muscles.

4. The name, Pelecypoda, means "hatched-footed". It refers to the shape the foot assumes during movement. After the foot is extended into the sand, it is spread out to form a hatched-shaped anchor. Then when the muscle of the foot contracts, the clam is pulled rapidly into the sand or mud.

5. A clam usually remains partially buried with its valves partly opened and its siphons extended into the water.

   a. Water is brought into the mantle cavity through the incurrent (ventral) siphon.

   b. Water passes up through the gills and then past the anus, where wastes are excreted. The water then passes out through the excurrent (dorsal) siphon.

6. As water passes over the gills two processes occur:

   a. Oxygen diffuses in and carbon dioxide diffuses out. (Respiration)

   b. Small particles of organic matter stick to a thin layer of mucus on the gills.

      (1) Cilia on the surface of the gills carry the mucus up to the dorsal surface. Here the cilia beat forward to carry mucous strands and trapped organic matter to the mouth.

          Note: Animals which feed in this way are called filter feeders or mucus feeders.

      (2) From the mouth, food passes through a short esophagus (gullet) to the stomach, which is connected by ducts to large digestive gland (liver), which is green. Enzymes secreted by the digestive gland digest food in the stomach.

      (3) The intestine begins from the ventral side of the stomach, passes through the heart, where absorption takes place. It finally ends at the anus, which opens near the dorsal (exhalent) siphon. Feces are carried away in the outgoing current.

7. The clam has an open circulatory system. (This means that blood is in blood vessels only in the vicinity of the heart.) Most invertebrates, if they have a circulatory system, have one of this type. The Annelids, plus all vertebrates, have a closed circulatory system. This means that blood is in blood vessels at all times.

8. The blood is colorless.

9. Nutrients and oxygen are carried by the blood to all parts of the body.

10. Carbon dioxide is transported to the gills where it is exchanged for oxygen. Liquid nitrogen wastes are transported to the kidney, which removes them and allows them to pass out the excurrent siphon. (Both carbon dioxide and liquid nitrogen wastes are formed as the individual cells in the clam's body burn food; that is, combine the food with oxygen.)

11. Three pairs of ganglia are present:

    a. One pair near the anterior adductor muscle.
    b. A second pair near the posterior adductor muscle.
    c. One pair near the foot region.

12. The edges of the mantle are provided with sensory cells, which are probably sensitive to contact and light.

13. Reproduction in the clam:

    a. Sexes are usually separate, although a few are hermaphroditic.
    b. Reproductive organs are situated in the foot.

14. A generalized life cycle:

    a. Sperms leave the male clam by the excurrent siphon. They are picked up by female clams by the incurrent siphon.

    b. Sperms are carried to the eggs. Fertilization occurs. The zygote becomes a free swimming larva, which leaves the mother via the excurrent siphon.

    c. The larva settles down to become an adult clam.

C. Example 2: Oysters

1. Some have reached a length of 1 meter.

2. The adult oysters are unable to move about, because their left valve is attached to some solid object or another oyster shell.

3. They have no foot.

4. A single oyster may deposit up to one half billion eggs in one season. Only a small percentage ever become an adult oyster.

5. Pearls are sometimes found in our common oysters, but best occur in pearl oysters of the tropical seas. The pearl is the result of an injury to the mollusk caused either by an organism or a foreign particle which embeds itself in the fleshy part of the oyster and causes an irritation. The irritation stimulates the mantle to deposit layer upon layer of nacre (pearly substance) around the intruding body to form the pearl. The amount of deposition is directly proportional to the degree of irritation. The mollusk requires from 3 to 4 years to form a good-sized pearl, and 7 years to form large ones. Pearls are colored cream, pink, rose, grey, white, bronze, black, lavender, blue, yellow, brown and green. Because the inner layer of an oyster's shell is composed of the same material that the pearl is made of, it is called mother of pearl. (Note: Pearls have also been found in fresh water clams. Some have been sold up to as much as $2,000.)

D. Example 3: Scallops

The only part of the scallop that we eat is its single adductor muscle. Unlike the clam, it has only one muscle holding the valves closed. Pecten irradians is the Atlantic bay scallops that we enjoy in New England.

E. Example 4: Mytilus edulis (edible mussel)

Found on top of rocks on rocky shores in the lower littoral zone. Byssus threads help this organism to adhere to objects.

F. Example 5: Teredo (Shipworm)

1. A shipworm feeds on wood particles and minute organisms. They burrow into the bottom of wooden ships, into wharfs, piles, etc., weakening and sometimes destroying them.

2. It is a bivlave, but the two shells which are used for boring enclose only a very small part of the anterior end of the body.

3. Every year, these mollusks do millions of dollars worth of damage to wooden wharf pilings and to ships.

IV. Class Cephalopoda (Cephalopodes) (head-foot)

A. Characteristics of the Class: Cephalopoda:

1. Head is large.

2. Foot is modified into tentacles which project from their heads and which are used for walking or food-getting.

3. Have no external shell (except for the chambered nautilus). Some have an internal shell. (The octopus has no shell.)

B. Example 1: Squid (Loligo pearlii is the species that is common along the eastern coast of North America from Maine to South Carolina.)

1. The foot consists of 10 arms bearing suckers and a siphon (funnel).

2. The arms are used for capturing prey.

3. The funnel is the principal steering and locomotion organ. If it is directed forward, the jets of water forced through it propel the animal backward; if it is directed backward, the animal is propelled forward. (This is the jet propulsion principle.)

4. The mantle in the posterior region is extended into triangular fins which may propel the squid slowly forward or backward.

5. It has two powerful chitinous jaws in the pharynx and a radula which consists of 2 rows of chitinous teeth. (Chitinous means hard.) These teeth tear up the food being drawn across it.

6. Above the rectum is an ink sac with a duct which opens near the anus. This is for protection. When the squid is attacked, it emits a cloud of inky fluid through a siphon to provide a "smoke screen" for its escape. It is also thought that the ink temporarily paralyses its enemy's sense of smell.

7. It has an internal shell.

8. Sense organs:

   a. Two very highly developed eyes. The eyes are similar to that of a vertebrate. It contains a retina, lens, cornea, pupil, iris, etc.

   b. Two statocycts (a vessel con taining a calcareous concretion - a pebble-like structure) is present. They seem to have something to do with its sense of balance.

9. The nervous system consists of a number of ganglia, most of which are in the head.

10. Sexes are separate.

11. They are famous for their color changes. Pigment cells filled with blue, purple, red, and yellow color are present in the skin. These color changes occur as rapidly as if they were blushing.

12. Some squids have luminescent organs.

13. The giant squid is the largest living invertebrate animal known, sometimes weighing as much as 2 tons. Near the coast of Newfoundland, giant squids are occasionally seen. They may be 15 meters or more in total length, with arms as large as a man's leg, and suckers as big as teacups.

C. Example 2: (Octopus Devilfish)

1. Live in dark crevices and in coral reefs.

2. Usually found in warm waters of the tropics rather than cooler waters of the temperate regions.

3. As a rule octupuses are timid creatures.

4. Most of them are not large enough to harm a human being; however, the giant devilfish of the Pacific reaches a diameter of 8.5 meters and are dangerous.

5. They have no shell.

6. They move by jet propulsion, or move along the bottom using their tentacles.

7. They can change color like squids.

8. Eggs are laid in jellied clusters on rocks. The young emerge as miniature adults.

D. Example 3: Cuttlefish (Sepia)

1. Has a short oval body bordered by fins that are usually united behind.

2. It possesses 10 arms, 2 of which are much longer than the others.

3. Its internal calcareous shell is the cuttlebone that we often see hung in bird cages.

4. Its ink has provided sepia (brownish-red pigments of artists).

E. Example 4: The chambered Nautilus (Nautilus)

1. The chambered nautilus has a shell that is coiled like a watch spring and is divided by cross walls into a series of compartments.

2. A new and larger chamber is built when the old compartment is outgrown; and a new wall is secreted (by the mantle) behind it. Each new successive chamber is larger than the preceding one.

3. All chambers, except the last, contain gas (mostly air with more nitrogen and less oxygen that is found in atmospheric air), which is secreted by the siphuncle (a tubular extension of the visceral hump that extends through perforations in the middle of the septa-partition).

4. The head bears 60 to 90 arms without suckers.

5. The nautilus resembles some of the extinct cephalopods.

V. Class: Amphineurs

A. Characteristics of the Class: Amphineura

1. There is an elongated body with a reduced head.

2. No tentacles.

3. Many forms have a shell composed of 8 plates.

4. They are the most primitive of all the mollusks.

B. Example 1: Chiton (Chaetopleura apiculate is the species found around here.)

VI. Class: Scaphopoda

A. Characteristics of the Class: Scaphopoda:

1. Body elongated and enclosed in a tubular shell, which is opened at both ends.

2. Gills are lacking.

3. All are marine.

B. Example 1: Tooth shells

VII. Importance of the Mollusks

A. Harmful

1. Slugs are sometimes injurious in greenhouses and gardens.

2. Shipworms (Teredo) burrow into the bottom of wooden ships, into wharfs, etc. weakening them and sometimes destroying them.

3. Some of the larger octopuses (octopi) have the reputation of killing human beings.

4. The giant clam (Tridacna) of the tropics, which may reach a length of 1.5 to 1.9 meters and weight 225 kg,is feared by pearl divers, because of the possibility of having a foot caught in the vise-like valves.

B. Beneficial

1. Food

a. Mya arenaria - soft shelled clam (steamers) (long necks)
b. Venus mercenaria or Mercenaria mercenaria - quahog

(1) The small size is called little necks.
(2) The medium sized is called cherrystones.

c. Spinula (Mactra) solidissima (Surf clam) - used by some in making chowders.

d. Pectan irradians or Aequipecten irradians - Atlantic Bay Scallop.

e. Placopecten magellanicus - Atlantic deep sea scallop.

f. Mytilus edulis - edible mussel, blue mussel.

g. Crassotrea virginica - Eastern oyster.

2. Other common Mollusks which may be used for food in certain parts of the world.

a. Ensis directus - Atlantic razor clam.
b. Littorina littorea - common periwinkle.
c. Busycon caricum - knobbed whelk.

3. Miscellaneous

a. Many people prefer to eat octopuses to oysters.
b. Abalone is eaten on the west coast.
c. Squids, cuttlefishes, and octopuses are eaten in southern Europe.
d. Only the large adductor muscle of the scallop are eaten. The rest of the body is not eaten.
e. Pearls are formed in pearly oysters (Maggaritifera) in Ceylon, India, Japan, and Northwest Australia. Pearls also occur in common oysters and in clams but are seldom of high value. The pearly layer of the clam shells are often used in making buttons.

UNIT IX

MARINE WORMS

Field Activities

1. Collection
2. Identification
   a. Ribbon worms
   b. Clam worms
   c. Trumpet worms
3. Importance and peculiarities pertaining to each, such as autotomy in ribbon worms.

Classroom Activities

Objective:

Study of marine worms using Nereis as an example.

Introduction:

Nereis and other members of this group of annelid worms have body appendages known as parapodia that enable them to swim through the water or push themselves along the substratum. Parapodial activity is synchronized and waves of movement sweep along each side of the animal. The setae extending from each parapodium aid in locomotion.

Lab I. Nereis - O_2 Uptake

Materials:

One gallon of sea water. One-half dozen live sea-worms (collect or purchase at bait shop). Two one-liter jars with lids. Chemicals or kit for dissolved O_2 test.

Procedure:

Weight six sea worms and put aside.
Aerate over two liters of the sea water by splashing it back and forth in a gallon jar. Fill the first liter jar to overflowing with the aerated H_2O. Cap well. Put 6 sea worms into the second jar. Add aerated water to overflowing. Cap well. Record the time. Check the amount of O_2 in another sample of sea water and assume that it is equal to that in both liter jars at time "zero".

After one hour, determine O_2 content of both jars in ml per liter. Use the first jar to correct the readings of the second jar, the one containing the worms.

Data:

1. Weight of worms _____grams

2. Ml of O_2 consumed per hour _____ml O_2/hr.

3. Temperature of water _____^{o}C

4. Equivalent O_2 consumed by 100 gram of sea worms at this temperature. _____ml

5. Corrected for STP

$$_____ml \times \frac{273^{o}C}{273^{o}C + \text{temp. in degrees Centigrade of water}} \times \frac{\text{Today's atmospheric pressure}}{60 \text{ mm Hg sea level}}$$

6. Assuming that each liter of O_2 burns 1.3 grams of glucose, the amount of glucose burned each hour by 100 grams of worms is _____ grams, at a temperature of _____^{o}C.

7. If each of these grams of glucose (from #6) contains 3.8 calories, then 100 grams of sea worms uses _____calories per hour at _____^{o}C.

8. The efficiency of the conversion into ATP is about 30-40% effective. Results_____.

Lab II. Pulse Rate of Nereis

Purpose:

To study the effects of temperature on "pulse rate"

Materials: Nereis, finger bowl, sea water.

Procedure:

1. Obtain a clamworm, Nereis virens.

2. Submerge the worm in sea water that has been precooled. What is the temperature?

3. The dorsal blood vessel fills and empties once for each contraction of the worm's aortic arch series or elastic blood vessels. This is an excellent opportunity to study the "pulse rate" of the worm. The dorsal blood vessel can be seen as a dark wavy line under the skin and it disappears when emptied by the action of the aortic arches or elastic blood vessels. Careful observation is necessary, but it can be seen with the unaided eye.

4. Make three recordings of "pulse rate per minute (p/min)" of the worm in cooled sea water. Calculate the average pulse rate. Make all recordings in tabular form.

5. Make three more recordings of pulse rate in room temp. sea water. Calculate the average pulse rate.

6. Make three recordings of pulse rate in sea water that has been warmed to 30°C by your adding warm water. Add this warm water slowly until the desired temp. is reached and then allow the worm to adjust for a few minutes before beginning your recording. Calculate the average pulse rate.

7. Using your recorded data plot a graph of pulse rate vs. temperature.

SUPPLEMENTAL PROJECT

VOLUME CONTROL IN ESTUARINE WORMS

Introduction:

There are two responses to osmotic stress. An animal which may change its body fluid concentration in response to its environment is called an osmo-conformer. When internal concentration remains relatively constant (osmotically stable) - called OSMO-regulaters.

Procedure:

1. Prepare various concentrations of sea water. Normal sea water is 100%; try 20%, 40%, 60%, 80%, 100% - to 160%.

2. Each of the team members should select one of the concentrations, using a marine worm.

3. Measure each worm by volume displacement in normal salt water.

4. Weigh each worm - blot it gently first.

5. Place worms in different concentration of sea water.

6. Reweigh all worms 3 X times at 2 hour intervals (6 hrs. good time).

7. At the end of the time - measure each worm again by volume displacement each in its own concentration and also by weight.

8. Return all worms to normal salt water for one hour - measure volume and weigh again as in #7.

9. Record weight changes $\frac{10}{20}$ $\frac{\text{increased 10 gm}}{\text{gm - initial weight}}$ = .5 X 100 = 50% increase in weight.

10. Record any volume changes again in % using initial displacement as 100%.

11. Get entire class results - get average so they can be plotted on graph - Do weight and volume.

12. Record assumptions.

SUPPLEMENTAL INFORMATION

MARINE WORMS

I. Phylum: Platyhelminthes (Flat Worms)

A. Characteristics of the Phylum: Platyhelminthes:

1. Body flat and ribbonlike.

2. No true segments.

3. No body cavity (coelom).

4. Nervous system is composed of two longitudinal nerves, which are connected by transverse nerves, giving the nervous system a ladder-like appearance.

5. The body consists of three layers of cells:

a. ectoderm (outer layer)
b. mesoderm (middle layer)
c. endoderm (inner layer)

B. Example 1: Bdelloura candida

Free-living. Commonly found on the gills of horseshoe crabs. This is an example of commensalism not parasitism.

II. Phylum: Annelida (Annelids) (Segmented Worms):

A. Characteristics of the Phylum: Annelida

1. Segmented worms with a body cavity (Coelom) separated from the digestive tube.

2. The "brain" is on the dorsal side of the anterior end.

3. A nerve cord runs throughout the body on the ventral side.

4. Has a closed circulatory system.

5. Body wall contains circular and longitudinal muscles.

6. Found in the soil and in freshwater; however, most are marine.

B. Example 1: Neanthes (Nereis) virens (clam worm) (sandworm)

1. Lives in tubes (burrows) in the sand or mud of the seashore between regions of high and low tide.

2. By day it rests in its burrow, but at night it extends its body in search of food. It may even leave its burrow.

3. Other worms and small sea animals are seized by the sharp horny jaws and are eaten.

4. Each segment bears a pair of appendages called parapodia, which have a number of setae (bristles) on them.

5. See page 57 of Golden Nature Guide, Seashores.

C. Example 2: Arenicola cristata (lugworm)

1. Burrowing animal like the earthworm.

2. Length from 15-20 cm.

3. Lives well below the surface and feeds by extracting organic matter from fine sand and debris that it take in as it burrows along.

4. See page 57 of Seashores.

D. Example 3: Clymenella torquata (bamboo worm) (jointed worm)

1. It uses its tail to clean out tube. The tail is like a plunger.

2. The rate of turnover of materials is very fast.

3. The segments are longer than they are wide. Each species has a definite number of segments.

4. There were certain mud flats where all of these worms were orange; on other flats they were all green. The different colors were due to the break-down products of what the animal was feeding. In order to prove this, posterior ends of orange worms were cut off and placed on anterior pieces of green worms. The result was that the entire worm turned green. The same procedure was repeated with the green worms with the result that they turned orange.

E. Example 4: Amphitidice oroata (ornate worm)

1. Have tentacles in head region.

F. Example 5: Caetopterus pergamentaceus (parchment worm)

1. Lives in U-shaped tubes in muddy flats.

2. Water is sucked in at one end, bringing oxygen and plankton food. Wastes are discharged at the other end.

3. The tube is built of a tough membrane. Male and female crabs may live in these tubes.

4. The tubes can be seen dotting the bottom of shallow water at low tide.

5. They are about 15 cm long.

6. They have a 17-minute feeding cycle:

   a. Filter.
   b. Roll up mucous bag thus ejecting particulate matter.

7. See page 57, Seashores.

G. Example 6: Pectinaria (Cistenides) gouldi (Trumpet worm) (Ice cream cone worm)

1. About 5 cm long.

2. They build their own conical tube out of grains of sand. This resembles a sugar cone.

3. Have plume-like appendages for respiration and gathering food.

4. Have curbed appendages to help them dig.

5. Refer to page 57, Seashores.

H. Example 7: Spirorbis borealis (spirorbis)

1. A coiled worm which lives in a coiled tube.

2. Very often found on seaweed.

I. Example 8: Lepidonotus (Scale worm)

1. Live under rocks.

2. Eats hydroids and sponges. Note: It is one of the few animals that will eat sponges.

UNIT X

NAVIGATION

Objective:

To enable students to plot a course, to understand navigational aids, and to prepare for a safe cruise.

Procedures:

I. The Magnetic Compass

Discussion:

One of the oldest and yet one of the most useful tools of the modern navigator is the magnetic compass. No boat which will ever be out of sight of its originating point, whether due to distance or poor visibility, should be without one. Traditionally sailors had to learn to "box the compass" or, in other words, be able to name all the Cardinal Points (North, East, South, and West) as well as the many intermediate points (Northeast, North by Northeast, etc.).

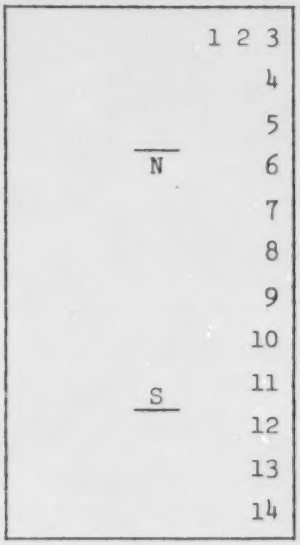
In our own time, however, this practice has given way to the much simpler method of describing directions in terms of degrees based upon the 360 degrees of the circle. Thus:

360° (or 0°) = North
090° = East
180° = South
270° = West

The compass depends upon the attractive force exerted by the magnetic north and south poles for its direction-seeking action. However, these magnetic poles are not located in the same positions as their corresponding geographic or "true" poles. Since the longitude lines used on charts and maps are laid out in terms of the geographic poles it can be seen that a line drawn on such a chart from a direction indicated by a compass will show this discrepancy. The difference between these "true" and "magnetic" directions is called variation. Variation is the angle between "true north" and "magnetic north" and is expressed in degrees. Variation is different all around the earth and is changing very slowly. In the area of New England variation is about 15°W. In the area of California it is 15°E; through Indiana it is zero. This means that the compass is pulled away from "true north" in the amount of 15° to the west. If variation is west you must subtract the appropriate number of degrees from any magnetic or compass reading in order to determine the "true" direction. If variation is east you must add the appropriate number of degrees to compass readings.

Exercise: The Magnetic Compass

Place a bar magnet on top of a sheet of plain white paper as shown in the diagram. Position your small compass in the locations indicated and in the order suggested. After the compass needle has come to rest, make a mark at either end. After removing the compass, connect the marks. After all the marks have been connected as pair, see if you can find a pattern in which several of the marks can be connected head-to-tail in order to form an overall geometric pattern. The magnet can be shifted to represent different variations.



Note: You will probably not be able to fit your compass in as many locations as indicated. Follow the pattern none the less.

On the basis of the above information fill in the following:

TRUE BEARING	VARIATION	MAGNETIC BEARING
150°	015°W	
	015°W	270°
075°		090°
	005°E	180°
220°	005°E	
065°		072°

It is recommended that students take bearings and actually fix their own position on a shoreline.

II. Chart Symbols---Using a Marine Chart

Discussion:

Marine charts have become indispensable to oceanographers, fishermen, professional seamen, and pleasure boat operators alike. Their history most likely goes back to the day that the first mariner, using a floating log, scratched the pictured description of a hidden harbor or underwater reef into the beach sand for the edification of a fellow voyager. Since that time, they have evolved into our modern-day Coast and Geodetic Survey (now, National Ocean Survey) charts which are based on accurate surveys, show extremely detailed information, and are beautifully printed.

Procedure:

In this activity you will work with a training chart (1210 Tr). On the back of the chart you will find an explanation of the symbols used, and these will enable you to answer the questions.

Methods:

Using a Coast and Geodetic Survey chart, answer the following questions:

1. What color is land which is permanently above water?

2. What color is land which is exposed at low tide?

3. What color is used to indicate relatively shallow water?

4. What color is used to indicate deeper water?

5. In what units is the depth of water shown?

6. What is the scale used in making this chart?

7. Explain what scale means.

8. What map projection was used to produce this chart?

9. At each of the following locations, designated by latitude and longitude, describe the object found. Include the following information: The kind and number of the object (e.g., buoy); its color, if any; size; characteristics of light(s), if any; type of sound produced; electronic aids (e.g., radio beacon); and any special characteristics indicated.

a.	41° 31.6' N	71° 23.9' W
b.	41° 21.0' N	71° 34.5' W
c.	41° 26.1' N	71° 26.2' W
d.	41° 16.6' N	71° 24.0' W
e.	41° 21.7' N	71° 28.9' W
f.	41° 25.6' N	71° 23.4' W
g.	41° 27.8' N	70° 51.2' W
h.	41° 23.4' N	70° 36.8' W

A suggested list of headings:

Lat. Long. Obj. # Color Light Char. Sound Electr. Aid Spec. Char.

Teacher Note: List various objects on charts you are using. You can get information about obtaining training charts from any local Power Squadron Officer or Coast Guard Station. It is suggested that you ask the students to locate about six to eight aids as described by your directions.

10. What is the usual color of nun buoys?
    How are they numbered?

11. What is the usual color of can buoys?
    How are they numbered?

12. Examine the entrance to a harbor. What do you notice about the arrangement of the buoys which mark the channel?

13. Make a sketch showing the actual appearance of nun and can buoys.

III. Simple Course Plotting

Discussion:

One necessary skill that a marine biologist or oceanographer should possess is the ability to "find his way" on the ocean where there are no streets and numbers, and familiar landmarks are scarce. The visible landmarks on the ocean are buoys and shore-based structures, such as lighthouses and other prominent features. The buoys have special characteristics (shape, color, number, and light or sound) that give the navigator information as to how to proceed relative to them. This unit assumes a familiarity with buoy characteristics. (See Chapman or other similar reference for this information.)

Materials:

Marine charts (Xeroxed portions of charts serve well for classroom work.)

Equipment:

Sharp pencil
Ruler
Protractor (Course plotters of various types normally used by navigators are not necessary, but may speed up calculations when their use is understood.)

Method:

1. Course Direction. A line must be drawn on the chart from the point of departure to the destination. (Needless to say, you must know where you are, to begin with.)

The direction of this course line must be determined with reference to the chart north, called true north. This may be done as follows:

(a) Extend the course line until it crosses a meridian or a parallel;
(b) measure the angle the course line makes with the meridian or the parallel;
(c) calculate the angle the course line makes with true north.

(Note: The meridians are oriented from true north, 0^{o}, to south, 180^{o}. The parallels are oriented from east, 90^{o}, to west, 270^{o}.)

Correct the course from true north to magnetic north so that you can follow it with the ship's compass. (The compass north, called magnetic north, is not the same as the chart north and, in this general area, is 15^{o} west of true north.) The correction is made as follows: (a) Find the compass rose on the chart; the magnetic variation is printed in the middle. The variation can also be seen as the difference between true north and magnetic north on the compass rose. (b) There are some rules that can be used in changing from chart to compass and vice versa. They are listed below. However, by inspecting the difference on the compass rose, the process can be reasoned out. When going from chart to compass, if the variation is west, add: if the variation is east, subtract. When going from compass to chart, if the variation is west, subtract; if the variation is east, add. (c) In this case, since the variation is west and we are going from chart to compass, the variation is added to the figure for the course based on true north. The resultant figure is the course in degrees magnetic and is the course to be followed on the vessel's compass. (Note: The compass may have a deviation which is a compass "error" due to metal, electric currents, etc. on a particular ship that must be corrected for and assume that the compass is correct and there is no current.)

2. Course Distance. Lay the edge of a piece of paper along the course line on the chart. Mark off the distance from start to finish. Lay this out on the mileage scale on the chart and determine the total mileage. (Note: One minute of latitude equals one nautical mile. This relationship is often used as a scale to measure mileage.)

Example

You have just gone under the Jamestown Bridge and are at the light on the southern end of Dutch Island. Plot a course from this point to Whale Rock. (Use chart 1210 Tr)

1. Draw a line on the chart from the dot that represents the light at the southern end of Dutch Island to Whale Rock. Extend the line past the Jamestown Bridge.

2. Lay the protractor along the parallel of 41°30' and measure the angle from this parallel to your course line. This angle is measured at 102°.

3. Add 90° to your measured figure because the parallel pointing east was already at 90° from true north. This gives you 192°, which is your course in degrees true. (An alternate method would be to use the parallel rule and walk it to the nearest compass rose and read your true and magnetic course from the compass rose.)

4. Find the compass rose and look up the variation, which is 15° west.

5. Add the variation of 15° to the true north course at 192°, giving you a course of 207° magnetic. This is the course to be followed on the compass.

6. Lay the edge of a piece of paper along the course line and mark the start and finish. Lay this out on the scale of nautical miles and determine the mileage. It is 3.2 nautical miles. (An alternate method is to use a pair of dividers. Spread the dividers to the course length and use your parallels of latitude because every minute of latitude equals one nautical mile.)

Problems

1. What is the compass course and distance from Whale Rock to Point Judith Light?

   Answer: 223°, 5.7 NM

2. What is the compass course and distance from Whale Rock to Brenton Reef Light?

   Answer: 142°, 1.8 NM

3. What is the compass course and distance from Brenton Reef Light to Castle Hill Light, Newport?

   Answer: 43°, 2.5 NM

4. What is the compass course and distance from Sandy Point Light, Block Island to Nebraska Shoal Buoy "2"? (South of Matunuck)

   Answer: 015°, 5.5 NM

IV. Supplementary Piloting Problem

You may use any books or other references that you may have to solve the problem. This problem should not be done in groups or by a class together. Each individual should have a separate chart and do the work himself.

Materials:

Chart 1210 Tr
Dividers
Parallel rules or protractor

Method

Plot your courses on the 1210 Tr Chart and label them adequately. Use 15° for variation and compute the deviation from the following table, interpolating to the nearest full degree. Use 6 knots as the speed of your boat.

DEVIATION TABLE

Mag. Heading	Deviation	Mag. Heading	Deviation
000°	11°W	195°	14°E
015	7°W	210	14°E
030	4°W	225	11°E
045	1°W	240	6°E
060	2°E	255	2°W
075	6°E	270	10°W
090	8°E	285	16°W
105	9°E	300	19°W
120	10°E	315	19°W
135	11°E	330	17°W
150	12°E	345	14°W
165	13°E	360	11°W
180			

Point of Departure - A "fix" with compass bearing as follows:

281° (compass) to Radio Beacon south of Old Harbor Point(Block Island)
018° (compass) to Point Judith Light
319° (compass) to Sandy Point Light

Use 19°W for deviation in establishing this fix.

1. The TRUE bearing of the Radio Beacon is:

   (a) 262°, (b) 247°, (c) 252°, (d) 067°

2. The TRUE bearing of Point Judith Light is:

   (a) 344°, (b) 052°, (c) 164°, (d) 022°

3. The TRUE bearing of Sandy Point Light is:

   (a) 353°, (b) 173°, (c) 105°, (d) 285°

4. Your Position at this fix is:

   (a) 41° 12.1'N 71° 25.2'W
   (b) 41° 11.8'N 71° 25.0'W
   (c) 41° 16.1'N 71° 25.8'W
   (d) 41° 12.9'N 71° 27.5'W

   You depart this FIX at 0900 and head for Bell "1B1" north of Sandy Point Light.

5. When you arrive at Bell "1B1" you will have travelled _____ miles.

   (a) 8.35 (b) 7.95 (c) 8.45 (d) 8.10

   You now head for Bell "4" southwest of Point Judith Light.

6. 20 minutes after departing Bell "1B1" the water depth is _____feet.

   (a) 143 (b) 150 (c) 120 (d) 115

   When Nebraska Shoal Buoy "2NS" bears 299° (TRUE) you turn east and head for Whistle Buoy "2".

7. You will reach this change of course at _____

   (a) 1109 (b) 0948 (c) 1021 (d) 1112

8. When heading towards Whistle Buoy "2" you will steer _____ (compass).

   (a) 103° (b) 073° (c) 089° (d) 269°

   When Point Judith Light bears 008°T and Sandy Point Light bears 213°T you change course and head northeast for Brenton Reef Light Station.

9. The Compass Bearing of Point Judith Light at this course change is _____.

(a) 024° (b) 016° (c) 196° (d) 204°

10. The Compass Bearing of Sandy Point Light at this change is _____.

(a) 049° (b) 229° (c) 221° (d) 041°

11. At this change of course you are _____ miles from the Brenton Reef Light Station.

(a) 6.8 (b) 7.2 (c) 8.2 (d) 6.2

You continue on this course until 1229 when you change course and head northwest for Whale Rock Lighted Buoy.

12. The Bottom at this course change will probably be _____.

(a) rock (b) mud (c) hard (d) sticky

13. You should arrive at Whale Rock Lighted Buoy at _____.

(a) 1249 (b) 1247 (c) 1251 (d) 1253

14. This last course is _____ miles long.

(a) 2.4 (b) 2.2 (c) 1.8 (d) 2.0

15. The TOTAL Distance Run this day is _____ miles.

(a) 23.4 (b) 22.9 (c) 19.4 (d) 22.5

IV. Field Activity.

Have students plot a course to be followed on the bay.

PAGE 77 MISSING FROM DOCUMENT

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Equipment and materials required: Part II

A quantity of cold extract from Part A; 2 - 500 cc graduated cylinders; balance a quantity of fresh whole milk, cocoa, sugar; a double boiler and thermometer.

Procedure:

Mix together 5.7 grams of cocoa and 33.1 grams of sugar. Heat 500 cc of milk in double boiler to 160 degrees F. Add the desired amount of carrageenin extract. Add the cocoa-sugar mix. Heat for ten minutes with constant stirring, to 50 degrees F. Bottle and hold in refrigerator overnight. Examine on the following day.

Note:

Be sure to use one bottle of chocolate milk without extract for a control.

Suggested levels of carrageenin usage:

Volume of extract used	Conc. of carrageenin in milk
0	0
5 cc. of extract	150 ppm
7 cc. of extract	210 ppm
10 cc. of extract	300 ppm
15 cc. of extract	450 ppm

SUPPLEMENTAL ACTIVITIES

PROJECT: THE LIGHT AND DARK BOTTLE METHOD FOR DETERMINING GROSS PRIMARY PRODUCTION

Discussion:

In order to study the energetic relationships in any ecosystem, an accurate estimate of the amount of solar energy fixed by green plants must be known. It is this energy that supports all other organisms in the community. The light and dark bottle method utilizes the one-to-one relationship between oxygen production and carbon fixation in photosynthesis to predict the amount of energy stored as energy-rich carbon compounds by the producers in an aquatic ecosystem.

Procedure-Materials:

2-3 liter collecting bottle
calibrated lines or bridle
250-ml ground-glass-stoppered bottles (paint one black)
All those materials and reagents needed for a standard dissolved oxygen determination.

Collecting the sample:

It is essential that the sample be representative of the given depth in the water column and that all atmospheric oxygen be excluded from the collecting and sample bottles.

Lower the collecting bottle to depth and secure at least a two-liter sample. Bring the bottle to the surface and fill three 250 ml ground-glass sample bottles (two clear and the other black) by overflowing each several times to exclude water that was in contact with the air in the bottle.

Return one "light" and one "dark" bottle to the depth from which the original sample was taken by suspending them from a calibrated line. (A bridle designed for this purpose is handy but not necessary.) Immediately "fix" the remaining sample according to the directions given for dissolved oxygen determination (D.O.D.).

Leave the light and dark bottles at depth for two to three hours and return to the lab to determine the oxygen content of the original sample (the D.O. of the third sample bottle). After two to three hours, collect the remaining bottles and determine their dissolved oxygen content.

Calculations:

The first bottle that you analysed represented the amount of oxygen present in the water column at the beginning of your study. By comparison of this value with the data obtained from the light and dark bottles you can determine the amount of oxygen produced by the photosynthetic organisms in your sample over the time of the test period.

Let us refer to the first sample as Bottle "O" and the light and dark bottles as "L" and "D" respectively. The light bottle should show an increase in D.O. due to the action of photosynthetic organisms. This value, however, must be corrected for the oxygen consumed by the organisms present in the bottle. The D.O. of the dark bottle should decrease over the test period. No photosynthesis is occurring in this bottle and theoretically the same amount of respiration is taking place here as in the light bottle. Based on this assumption then we can see that the amount of oxygen consumed in the dark bottle should be equal to that consumed in the light bottle. Thus we can calculate the total amount of oxygen produced by the producers in the light bottle by adding the decrease in D.O. of the dark bottle to the increase in D.O. in the light bottle. This value is the gross primary production in the sample.

$$L - O = X$$

$$O - D = Y$$

$$X + Y = P \text{ or gross primary production}$$

This value, in mg O_2/l or in ppm, can be used directly to compare productivity -- at different depths, of different water columns, of the same water column at different times of the day or year.

In the literature, production is generally given in mg C/m^3. The following chemical relationship between fixed carbon and molecular oxygen can be used to convert mg O_2/l to mg C/m^3:

PHOTOSYNTHESIS

$$6\ CO_2 + 6\ H_2O + E \text{ -- } C_6H_{12}O_6 + 6\ O_2$$

Assuming a one liter sample at standard temperature and pressure

$$\text{mg C} = (\text{mg } O_2)\ \frac{(1 \text{ mole } O_2)}{(32\text{X}10^3 \text{mg} O_2)}\ \frac{(6 \text{ mole } CO_2)}{(6 \text{ mole } O_2)}\ \frac{(12\text{X}10^3 \text{mg C})}{(1 \text{ mole } CO_2)}$$

$$= (\text{mg } O_2)\ \frac{(12\text{X}10^3 \text{mg C})}{(32\text{X}10^3 \text{mg } O_2)}$$

$$= .375\ (\text{mg of } O_2)$$

Since one cubic meter contains approximately 10^6l we can convert mgC/l to mg C/m^3 by knowing that $mgC/m^3 = (\text{mg C/l})\ (10^6 l/m^3)$.

Additional Projects:

1. Determine the effect of light intensity on production.
2. Determine the effect of light quality on production.
3. Determine the effect of CO_2 concentration on production.
4. Determine the effect of temperature on production.
5. Determine the relative production efficiency of various phytoplankters under similar environmental conditions.

Referendes:

Heister, R. D. 1972. "Measuring Dissolved Oxygen in Water", Environmental Activities New Bulletin, C. E. Merrill, Columbus, Ohio. 1:1.

Macan, T. T. 1963. Fresh Water Ecology, Longman Group Ltd., London.

Nygarrd, G. 1955. "On the Productivity of Five Danish Waters", Verh. int. Ver. Limnology, 12:123-133.

PROJECT: PREPARATION OF HERBARIUM MOUNTS

Objective:

To prepare herbarium mounts of marine algae.

Discussion:

As a coming source of food for mankind, algae cannot be overlooked. At the present time seaweeds, giant kelp, are being collected and converted to flour. The Japanese are raising *Chlorella* to be used as a protein, fat, and vitamin supplement for their diets.

Materials:

plant press
plastic bags and ties
tags (white paper)

shallow pans or trays
probes
scissors
5 x 8 file cards or herbarium sheet
wax paper
cellophane wrap
newspaper
drying box
assorted algae

Procedure:

1. Remove a specimen from a numbered bag and record this number on 13 x 20 cm unlined file card.
2. To a shallow, flat pan add water to a depth of 6.5 mm.
3. Place the algae in the pan and tease it into flat position.
4. Select a representative portion to use as a mount.
5. Slip the card under the specimen and arrange the specimen into desired position.
6. Remove the mount by grasping a corner and raising gently to drain the water. A second method is to mount the paper on a floating board and place in water for mounting.
7. Place the mounted algae into a plant press using alternate layers of newspapers, the algae mount, wax paper, newspaper.

8. Place the press in a drying box or a hot dry place.

9. Change the newspaper and wax paper every 6-12 hours or as often as possible to at least once a day.

10. When the mount is dry, cover it with a sheet of wrap slightly larger than the mount and overlap the self-adhering portions on the back of the card.

11. Mount the preparation on a sheet of 21.5 x 28 cm stock and record collecting data from corresponding number records.

12. Secure dichotomous keys and/or refer to the school collection for identification. Record the following:

    Technical name-

    Common name-

    Family-

    Habitat-

    Locale-

    Description-

    Date-

    Collector

UNIT XII

SPONGES AND COELENTERATES

PROJECT: SPONGE STRUCTURE

Objective:

To study the structure of some common sponges and identify these sponges.

Materials:

preserved and living sponges	microscope	slides, coverslips
10% acetic acid solution	probe	sea water

Procedure:

1. Place a specimen of sponge A on a clean slide. Find the osculum and pores. Sketch and label. Examine a specimen of sponge A which has been cut longitudinally. Sketch and then label the spongocoel.

2. Place a small piece of tissue from sponge A in a drop of water on a slide. Tease apart the tissue, coverslip, and study under 100 and 400X. Sketch and label any structures or cells that you can identify.

3. Place a small piece of tissue from sponge A in a drop of 10% acetic acid on a clean slide. Coverslip and observe. If bubbles of a gas are evolved, this indicates spicules made of calcium carbonate, $CaCO_3$. The formulas and equation is as follows:

$$CaCO_3 + 2\ HC_2H_3O_2 \text{ -- } Ca(C_2H_3O_2)_2 + H_2O + CO_2$$

4. Examine and sketch sponge B. Then test a small bit of tissue with acetic acid as you did in procedure #3.

5. Obtain a fresh bit of tissue from sponge B and place it in a drop of sea water. Coverslip carefully and study under 100 and 400X. Sketch and label identifiable parts. Then set the slide apart on a piece of paper labeled Sponge B as you will examine the slide later.

6. Repeat procedures #4 and #5 with sponges C and D, setting aside slide preparations for later study. If slides appear dry, add sea water.

7. On the basis of your observations, identify the sponges using the key on the next page.

8. Examine the slides of sponges B, C, and D which have been set aside. Record your observations and suggest an explanation.

KEY TO COMMON SPONGES

1. a. Structure of sponge simple; tubular to urn-shaped; pale tan to whitish; spicules calcareous (test with acetic acid).

   b. Structure massive, fleshy, or spongy; encrusting or branching; color various; spicules siliceous.

2. a. Sponges in form of branching, cylindrical, pale tubes . . . Leucosolenia sp.

   b. Sponges in the form of little urns or vases, usually clustered, with fringe of spicules around the terminal asculum . . . Scypha sp.

3. a. Spicules smaller (less than 200 u in length) and conspicuously joined together by more or less spongin to form a network or a system of tracts; dermal skeleton absent . . . Haliclona

   b. Spicules larger (mean length greater than 200 u), arranged in loose tracts with little spongin or occurring at random; a distinct dermal skeleton present . . . Halichondria

PROJECT: SPONGE REGENERATION

Objective:

To observe the re-integration of sponge cells.

Materials:

Stentor dish, forceps, slides, coverslips, microscope, red beard sponge.

PROCEDURES:

Place a drop of sea water on a clean slide. Grasp a small piece of living sponge with forceps and squeeze the sponge into the water drop until the sponge pigment is barely visible in the water.

THE FOLLOWING STEPS MUST BE CARRIED OUT QUICKLY (30 seconds).

1. Discard the piece of sponge and remove any visible sponge fragments from the water drop.

2. Place a clean coverslip on the drop, and examine the sponge-cell suspension under the microscope. Note the distribution of cells under low power. Notice the degree, or lack of, clumping.

3. Observe under high power.

4. Careful observation should enable you to see the extension of delicate, transparent psuedopods.

5. Carefully place the slide, with coverslip in place, into a stentor dish of sea water.

6. Five minutes later, remove the slide from the culture dish. Dry the bottom surface of the slide and examine under the microscope. Look for signs of additional cell clumping. Draw and describe the clumping.

7. Return the slide to the culture dish and gently slip the coverglass off the microscope slide. This will allow unrestricted growth of the newly-formed colonies of sponge cells.

8. Re-examine the slide at intervals during the day and on following days.

9. During reorganization, small filaments may be seen extending to other cells. Upon contact with other cells, these strands withdraw, pulling other cells into small clumps.

SUPPLEMENTAL INFORMATION

I. Phylum: Porifera (Pore-bearing)

A. Characteristics of the Phylum: Porifera

1. Body in two cell layers, (outer layer called ectoderm; inner layer called endoderm) penetrated by numerous pores. Between the inner and outer layer, there is a jellylike substance called mesoglea.

2. "Skeleton" formed by silicious (containing silicon and oxygen as in quartz) or calcarecus (containing calcium carbonate) spicules or horny spongin.

3. Marine (salt water) or aquatic (fresh water) animals.

B. Example: Sponges.

1. How a Sponge Feeds.

a. Water containing food particles (diatoms, small protozoans, bacteria, organiz particles) and oxygen enter the ostia (incurrent pores).

b. The food particles are caught by the collar cells and are digested by the enzymes within the collar cells. (The collar cells are part of the endoderm.)

c. The digested food is absorbed by amebocytes (cells that look like amoebae). The amebocytes wander throughout the mesoglea, transporting food and oxygen to the other cells.

d. The amebocytes carry cell wastes (liquid nitrogen wastes which are the result of the burning of food in the cells) and carbon dioxide (also a product of burning food) to the collar cells for disposal.

e. The carbon dioxide and the liquid wastes go out to the osculum (excurrent pore) with the current created by the collar cells.

2. Reproduction in a Sponge

a. Asexual Reproduction

(1) Sponges may form buds, which are groups of cells that enlarge and live attached to the parent for a time, then break off and live independently.

(2) During periods of freezing temperatures or drought, groups of amebocytes and spicules become surrounded by a heavy organic coat. Now they are called gemules. When favorable conditions return, each gemule has the capacity to grow into another sponge.

(3) Sponges are able to regenerate missing parts. Because of this ability, sponge-growers are able to increase the number of sponges by cutting them in pieces. These pieces are placed in special growing beds. (Regeneration).

b. Sexual Reproduction

(1) Sponges are capable of producing eggs and sperms in the same individual; therefore, they are referred to as being monoecious or hermaphroditic. Note: organisms like man that are capable of producing either egg or sperm are referred to as being dioecious.

(2) In most cases there is cross-fertilization; that is, the sperm from sponge number 1 fertilizes the egg in sponge number 2.

(3) The sperms are shed into the water by way of the osculum and enter another sponge through its ostia.

(4) The sperms are ingested by the collar cells and are transferred to egg cells (located in the mesoglea) by the amebocytes.

(5) When the sperm unites with an egg, a zygote is formed.

(6) The zygote develops into a free swimming larva called the amphiblastula larva. This free swimming larva leaves the parent sponge by way of the osculum, swims around for a while, and finally settles down to become a young sponge.

II. Phylum: Coelenterata (hollow gut) (Coelenterate)

A. Characteristics of the Phylum: Coelenterata

1. Have a bag-like body of two cell layers. (Ectoderm, endoderm with mesoglea in the middle.

2. A gastrovascular cavity with one opening leading to the outside is present.

3. Tentacles are present with stinging capsules (nematocysts) in them.

4. Solitary or colonial forms.

5. Marine or aquatic.

6. Vary in size from being microscopic to over a meter in diameter.

B. Example 1: Hydra (a small freshwater form).

C. Example 2: Colonies of feeding polyps (Tubularia) is found on seaweed).

D. Example 3: Jellyfish

1. Jellyfish reproduce in a very peculiar way. What we call a jellyfish is the medusa or generation that reproduces sexually (by means of eggs and sperms). When the zygote is formed, it develops into a colony of feeding polyps similar to the members of example 2. Part of this colony reproduces asexually and forms medusae. Reproduction of this type is called alternation of generations.

2. Life cycle of Obelia

3. Other jellyfish:

a. Aurelia - very common around here.

b. Physalia (Portuguese-man-of-war)

(1) It is made up of colonies of polyps. It is not an individual organism like Aurelia. One polyp acts as a float. All other polyps are attached to this float. Some polyps digest food, others catch food, and other specialize in producing gametes (either eggs or sperms).

(2) Usually they are found in the tropics or semi-tropics. Their tentacles can be up to 15 meters long.

(3) One species of fish, Noemus, swims about among the tentacles without being harmed. Noemus darts out to grasp a small food animal and hastens back amid the safety of the Portuguese-man-of-war's tentacles to devour it. The tentacles protect the fish, and the particles of food not eaten by the fish are engulfed by the Portuguese-man-of-war. (This is an example of mutualism).

E. Example 4: Coral (Formed by tiny little polyps which secrete limestone cups into which they can retract).

F. Example 5: Sea Anemones (Metridium is the genus that is common around here.) They are very often called flower animals, because they look like beautiful flowers. Because of their bright colors, they attract many animals, which they paralyzed by stinging them with their nematocysts.

G. Example 6: Sea fans - found in tropical waters. (See page 52 of Golden Nature Guide, Seashores.)

UNIT XIII

POLITICAL AND SOCIAL INVESTIGATIONS

Objectives:

1. To acquaint students with agencies which deal with marine environmental problems.

2. To make students aware of the processes through which environmental legislation must pass.

3. To prepare students for their roles as citizens and voters.

Introduction:

Social and politial investigations make excellent winter-time activities for students. Students will find that investigation into any of the following topics will prove quite interesting:

1. Coastal Resources Management Council (A division of the R. I. Department of Natural Resources created to insure ecological use of shore areas).

2. Coastal Resources Center (a division of the U.R.I. School of Oceanography).

3. Various local governmental agencies.

4. R. I. Department of Natural Resources.

5. R. I. State Department of Health.

6. Wetlands Protection Acts.

7. Save the Bay.

8. Ecology Action.

9. Oil company public relations departments.

10. Electric company public relations departments.

11. Environmental Protection Agency.

12. 200 mile fishing limit.

The following series of activities pertain in particular to governmental control over water pollution.

Activity I: State Government Model

A. Introduction

This is an activity which could follow one in which the state government structure in the area of water pollution has been studied. It is assumed that the students have been impressed with the complexity of governmental operations; the duplication of efforts; the inefficiencies of the various bureaus, commissions, boards, etc. It is, therefore, anticipated that the students might wish to develop their own organizational plan for water pollution control. The students may then wish to make suggestions to their legislators or to special appointed task forces so that the immediate serious problems might be solved by minimizing the usual red tape and delays.

B. Questions

1. Lead to the activity by asking:

   a. Why does it take so long to get things done?
   b. Why is it so hard to get questions answered?
   c. Are you surprised by the complexity of the structure of the state government?
   d. Do you think the present one can operate efficiently and effectively?
   e. Do you notice that various aspects of the water pollution program come under different agencies?

2. Initiate the activity with questions such as:

   a. Can you name all the people and organizations that might be concerned with water pollution?
   b. Do you think that certain areas are not covered?
   c. Do you think that efforts are being duplicated?
   d. Do you think that you can come up with a better type of organization?
   e. What are some desirable changes that are in order?
   f. How do you think that the changes can be brought about?

3. Continue the activity by asking:

   a. Now that you have developed a plan which you think is more efficient and effective, do you wish to pass this on to your legislators?
   b. Can you name some other individuals and organizations that might be interested in your plan?
   c. If no party or parties show any interest in your plan, do you wish to revise or alter the plan?

4. Evaluate the students' efforts with questions such as:

   a. Did this activity interest the students?
   b. Did they wish to extend the study?

c. Did they really feel that they were making a contribution to the solution of the problem?

C. Equipment

No equipment is needed. Various booklets on the structure of state governments - from the state in which the school is located or (if a boarding school) from the home states. Typewriter, duplicating, or copying machines are in order.

D. Procedure

1. After students have expressed their dissatisfaction with the present system of water pollution control, suggest (or have the students suggest) that they develop a better system which would more efficiently coordinate all the agencies, commissions, etc.

2. Have the students block out a table of organization.

3. Compare the students' plan with the one proposed by their state.

4. Suggest follow-up by writing letters and enclosing the plan to legislators and others who might be interested.

5. Students may be encouraged to make charts and posters explaining what they hope to accomplish.

Activity II: Anti-pollution Laws

A. Introduction

This activity is designed to determine what circumstances in a given area allow cases of obvious pollution to continue. While it is true that the time gap between creation and enforcement of laws is one of the primary causes, this is not always the case. If anti-pollution laws do exist, it may be that a gap also exists between what is considered to constitute pollution and what legally constitutes a case of pollution. In other words, both legal and illegal polluters have been found to exist.

In order to make such determinations, the students are required to wade through many legal documents as well as carry out interviews.

B. Questions

1. Lead to the activity by asking:

a. Why isn't something being done about citing a local polluter?

b. How can you determine the legal status of an industry?

2. To initiate the activity ask:

   a. What agencies (public and private) are directly concerned with industrial pollution in your river basin?

   b. Which ones make the regulations?

   c. What are they?

   d. What people should be contacted for information? Local? State? Federal?

   e. What questions do you want answered? For example, is there a water quality standard in your state?

3. To continue the activity ask:

   a. What types of testing have been done?

   b. Should you make tests of your own?

   c. Who interprets the results of the testing?

   d. What is the mechanism for reporting violations?

   e. How do you survey local industry?

   f. What steps are being taken toward sewage abatement?

   g. Who is responsible for enforcement of water pollution regulations?

4. To evaluate the student consider:

   a. What types of background material did the student gather?

   b. Were the questions formulated in advance of personal contact with resource people?

   c. Was the plan of attack well planned and viable?

   d. Can the student differentiate between legal and illegal pollution practices?

   e. Is the student aware of public recourse that can be brought against the illegal industrial polluter and the steps in this process?

C. Equipment

No special equipment is required unless the students do testing in the field.

D. Procedure

1. Select a site of obvious water pollution.

2. Determine the industrial or private persons who are contributing to the pollution.

3. Investigate the local, state, and federal agencies concerned with pollution in your area and determine what laws are now in existence.

4. Select one specific industrial polluter and secure background material on the corporation, i.e.:

   a. How is it polluting and to what degree (may be necessary to perform tests)?
   b. When did it begin?
   c. How many people are employed?
   d. What are its gross earnings?
   e. What responsibility does it feel it has?

5. If a violation is occurring, discuss the courses of action regarding it. You may want to do one of the following:

   a. Go to the corporation's management and ask about the responsibility to meet legal standards, past actions, and projected activities.
   b. Go to the local politicians about the specific corporation.
   c. Go to the relevant enforcement agencies with your data and attempt to find out what they are doing.

Activity III: An Elementary Investigation of Local Water Anti-pollution Programs by Interviewing Government Officials

A. Introduction

This activity could be used in classes to evaluate the evident effectiveness of the government to deal with water pollution. The students should become aware of and develop an interest in the local problems of their communities.

B. Questions

1. Lead to the activity by asking what are the water pollution problems in our community.

2. Stir interest by asking:

   a. Who are the people responsible for controlling these problems?
   b. Do they use the authority given them effectively?

3. The teacher may evaluate the activity by considering:

   a. What were the students' results?

b. What reasons were there for these results?
c. Were the students' questions well prepared?
d. Was the students' back-up knowledge sufficient?

C. Equipment

None is required.

D. Procedure

1. Find out a few problems in your community by reading the newspaper.

2. Determine which laws pertain to these problems.

3. Make up an outline of questions.

4. Set up the interview.

5. Record the results and your reactions by writing articles or reports.

Activity IV: Orientation Program For the Study of Water Pollution

A. Introduction

This activity is set up as a discussion for a group orientation study of water pollution. The group can be a traditional class. It could also be a community group (e.g., students from several high schools that do not offer a course in water pollution). The questions should stimulate the group into shaping a skeleton from which the leader can plan a study agreeable to all. It would be helpful to get through the whole activity in one session. However, the rate of progression must be determined by the group. Tape recording the discussion would have value; the group leader could use it as a reference in the future. The questions are set up under the precept that the group will be situated by a polluted body of water. Perhaps it will be the one the group decided to study. This natural setting should act as a motivating device, as seeing the problem would increase awareness and hopefully concern among the group.

B. Questions

These questions are to provide thought-provoking topics for discussion. The first three sections play a specific role in the progression of the orientation.

1. To lead into the activity - these questions are to "set the stage", to lead the group to concentrate on water pollution. They lead into the real investigation.

   a. What is pollution?

b. Can you identify by sight any pollution in this water?
c. Are natural things like leaves and twigs pollution?
d. How is a scientific approach to the problem relevant?
e. What can science tell us about the problem?
f. Can this information help us to solve the problem?
g. How can data and facts help us?
h. Why is a social approach important?
i. How can a social approach help to solve the problem?
j. How can public relations help with a commercial approach to fighting pollution?
k. On which commercial enterprises should attention be focused?
l. What type of public relations is important?
m. Reflecting recent months, pollution plays an important role in politics. How can politics influence pollution?
n. How can his outlook on pollution affect the fate of a politician?

2. To initiate the activity - the trend should be set in a meaningful direction at this point. Discussion now centers about the objectives of the group. These shall be recognized by covering the points to each numbered them question.

a. Should we study a specific body of water?
b. What would you like to find out about the pollution of this water:
   -chemical
   -bacterial
   -historical
   -aquatic life
   -public influence
c. Are we going to try to solve the pollution problem?
   -(apply what was discussed in A)
   -when
d. How shall we divide the group, if at all?
   -scientific
   -social
   -commercial
   -legislative
   -political
e. Whom shall we involve in fighting this pollution?
   -peers
   -family
   -community
f. What type of information shall we request, and what commercial enterprises shall we contact?
   -only water polluters
   -any polluters
   -research agencies
   -factories
   -small enterprises

g. What information shall we seek?
   -history
   -general information
   -a role we can assume now

h. What shall our group objective be?
   -(tie together what was discussed)

3. To continue the activity - now that the atmosphere is set and the group objective outlined, these questions focus on planning the group's activities. The extent of the use of the questions will vary, especially in the case of the high school students. Many will have to have been answered by other than the group in preparation of a type of contract, be it a community group

   a. Where shall we begin?
      -introduce limitations set by authorities, if it is necessary
      -frequency of group sessions
      -summarize B and make it concrete
      -independent work
      -funding
      -publicity

4. To evaluate the students'performance - these questions can be applied to a classroom situation if the need for an evaluation persists. If it is a community group, this evaluation may be unnecessary. The leader will have to evaluate a group of high school students if their schools request it. Evaluation may also be necessary if credit is to be given for the study.

   a. Did the group member help set a meaningful trend to the discussion?
   b. Did he (she) make specific personal objectives of the study?
   c. Did he (she) help with the setting of the group objective?
   d. Did he (she) introduce relevant discussion matters not included in the outline?

UNIT XIV

TIDES AND CURRENTS

Objectives:

To acquaint the student with the various characteristics of local tides and currents.

I. Current Bottles

Materials:

soda bottles - preferably the 300-350 ml, thick-walled returnable type with screw-on caps

7.5 x 13 cm cards

wax

Procedure:

Bottle preparation. First the cards must be marked. Be sure to use a soft pencil. Ink will rapidly fade in sunlight and these bottles are frequently exposed to weeks of direct light.

Be sure there are identifying marks for cards that are thrown from different locations. Nothing is more frustrating than to get a card back in the mail and not be able to find out where it was dropped off.

After the cards are marked and placed in the bottles, put the caps on as tightly as possible. The cap should then be dipped in hot wax and allowed to cool. CAUTION: hot wax is flammable and can also cause severe burns. It should be handled with extreme caution and under direct supervision of a teacher. After the wax has cooled, second, third, and fourth dippings are suggested. Be sure to make them brief, or else you will simply melt off the wax from the previous time. Allow the wax to cool between each dipping. If the wax you use is too brittle for a good waterproof seal, add a small amount of petroleum jelly to soften it up. After the bottles are sealed (you can spot check with a bucket of water), they are ready to be used.

When selecting a spot to throw your bottles, a few things should be kept in mind. (1) Try to select a place where there are definite currents, preferably headed offshore. If there is a mixture of local constantly shifting eddies and wind currents, it will be difficult to get any meaningful results. (2) Try to avoid throwing the bottles during a strong incoming tide. This usually results in your bottles being washed up very close to where they were thrown in. (3) Bottles thrown off during the outgoing tide, particularly near a river, can

often be carried a long way offshore. If you have access to a boat, your problems are greatly simplified because you can be selective in your drop site. (4) Throw off at least ten bottles from any given spot. Your chance of multiple returns is much better.

II. CONSTRUCTION OF A SIMPLE WAVE MACHINE

Objective:

Anyone can construct a simple wave machine with materials you can find around your house.

Materials:

1. Denatured alcohol (available at the drugstore).
2. Paint thinner.
3. 10 or 20 gal. aquarium, rectangular, or sediment tank, marine geology.
4. Blue food coloring or methylene blue indicator.

Procedure:

1. Fill 1/3 of the container with alcohol.
2. Add a few drops of blue coloring until the desired color blue appears. You may have to swirl the container until the coloring and alcohol mix.
3. Fill the container with paint thinner.

Alcohol has a greater density than paint thinner. Due to the differences in density of the two liquids, the alcohol (the heavier liquid) returns to its original bottom position as you tilt the container and make waves.

III. Current Velocities

Introduction:

Measuring the current velocity and flow of various currents can be a useful and exciting field lab. The experience can help develop such ecological concepts as the seasonal changes in the flow of water through a given marine ecosystem. A practical and relatively inexpensive current indicator for deep water was used by Pritchard and Burt while they were doing current studies of the Chesapeake Bay. The device consists of a biplane cross constructed of two sheets of 12.6 mm plywood, each of which is 1.2 meters wide and 9 meters high. The two sheets are assembled so that either panel bisects the other. A 14 kg weight is suspended below the apparatus to assure negative buoyancy.

Materials:

A Pritchard and Burt biplane cross current indicator.

Procedure:

Suspend the apparatus by a thin cable from an anchored boat. The top of cable should be elevated about 1 meter above the side of the boat. The biplane will swing in the direction of the current. The speed of the current can be computed from the angle of the suspending wire according to the formula:

$$v=(k)\ (\tan 0)$$

where v is the current velocity in knots, k is an empirically developed constant 1.04; and tan 0 is the tangent of the angle formed between the supporting wire and the vertical.

IV. "The 24-Hour Clock"

Since the tide table uses the 24-hour system, it is a good idea to be trained in its use. It will permit you to use it in the classroom lab situation and in the field.

You can convert a clock by numbering the extra hours, 12-24, on the clock face or around the margin. Do this to your bedroom clock. Remember standard time is used in most published reports and tables -- be prepared to acknowledge "daylight saving time".

The reason for the 24-hour clock is accuracy in reporting and in communicating -- the day begins at midnight and runs through a 24-hour period. 2400 hrs. June 10 = 0000 hrs. June 11. It is easily adapted for worldwide usage.

Exercise 1:

1. Conversion to 24-hour time - use four digits, convert the following.

2. Say time orally - expressed in 100's.

a.	8:15 A.M.	a. _____
b.	8:15 P.M.	b. _____
c.	11:00 A.M.	c. _____
d.	11:00 P.M.	d. _____
e.	2:30 P.M.	e. _____
f.	4:30 P.M.	f. _____
g.	12:00 Noon	g. _____
h.	12:00 Midnight	h. _____
i.	12:00 A.M.	i. _____
j.	12:00 P.M.	j. _____

Exercise 2:

1. Compute time differences - the first 2 digits = hours
   last 2 digits = minutes

2. Remember minutes over 60 must be converted to hours - if subtracting and you borrow from the second digit, you borrow 60 minutes.

Addition:		Subtraction:	
a. 0240		a. 1358	
+0120	_____	-0050	_____
b. 1040		b. 0911	
+1000	_____	-0119	_____
c. 2250		c. 0234	
+0120	_____	-0050	_____
d. 1100		d. 0120	
+0230	_____	-0300	_____

V. A Study of Tidal Ranges

Background:

This activity can be done along with another activity since it does not require 100 per cent of the student's time. The purpose of this experiment is to study visually the change in water level from low tide to high tide (or vice versa) at various regions (i.e. beaches, tidal marshes, mudflats). It can be done just about anywhere along the New England coast. For the best results, you should choose a day in which there is a large (spring) tidal range. Spring tidal ranges occur twice a month around the time of new and full moon. Whenever there is a spring tide the water rises higher at the time of high tide and falls lower at the time of low tide. Incidentally, these days are excellent for studying marine organisms that usually are not uncovered during most low tides. You should consult your local newspaper for the best day in which to perform this activity. In general, days with the largest tidal range occur when the low tide occurs around 7 A.M. and 7 P.M. or 8 A.M. and 8 P.M. This would mean that the tide would be high at around 1 A.M. and 1 P.M. for the seven o'clock lows and 2 A.M. and 2 P.M. for the eight o'clock lows. A tide table for such a day might look as follows:

High Tide	1:00 A.M.	Height 12.2 feet
Low Tide	7:12 A.M.	Height -.6 feet
High Tide	1:25 P.M.	Height 12.4 feet
Low Tide	7:37 P.M.	Height -.7 feet

Note:

1. The negative sign means that the low tide will fall .6 ft. (.7 ft.) below the mean low tide level.

2. There is approximately 6 hours 12.5 minutes between one high tide and its corresponding low tide.

3. There is approximately 12 hours and 25 minutes between the two consecutive high tides and the two consecutive low tides.

Field Equipment:

An inexpensive camera (i.e. Kodak Instamatic) that will take slides. Take prints if you prefer. Some people might prefer to use a Polaroid camera. A tripod on which to rest the camera is suggested but not absolutely necessary.

Field Assignment:

Divide students into groups of two and assign them to the specific areas that are to be studied. The teacher might wish to assign the students another activity that can be worked on between observations.

Procedure 1:

1. Arrive at the site to be observed (beach, tidal marsh, mudflat) at the time of low (or high) tide. Take a picture of the specific area that you wish to study.

2. Take subsequent pictures each hour from the exact same spot until the tide is high (low). In other words, you will have seven pictures as indicated in the example below.

   a. 8:12 A.M. (Time of low tide)

   b. 9:12 A.M. (One hour after the time of low tide)

   c. 10:12 A.M. (Two hours after the time of low tide)

   d. 11:12 A.M. (Three hours after the time of low tide)

   e. 12:12 P.M. (Four hours after the time of low tide)

   f. 1:12 P.M. (Five hours after the time of low tide)

   g. 2:35 P.M. (The time of high tide 6 hours 12.5 minutes after the time of low tide)

Procedure 2:

1. Take a picture of several beaches, tidal marshes, and mud flats at the time of low (high) tide.

2. Return to the exact same spot at the time of high (low) tide and take pictures from the exact same spot.

3. You will end up with a series of pictures or slides which show a given site at high and low tide. The slides can be flashed on a screen rapidly to give you almost the same effect as time-lapse photography.

Note:

1. If you begin taking pictures at the time of low tide, make sure that your site is not submerged during the time of high tide.

2. If you decide to try several beaches, pick one that slopes gently and one that slopes a great deal. You might try a rocky beach and a sandy beach as well.

3. In procedure 1, you may wish to decrease the interval of time between successive pictures from one hour to a half hour.

4. In procedure 2, you may desire to take a picture of the site half way between high tide and low tide.

UNIT XV

ECONOMIC INVESTIGATIONS

A. Plan field trips and/or guest speakers from the following:

1. Seafood Processing

Captain Milt's Dock
State Pier
Galilee, RI

Coast Canning and Processing Fish Co., Inc.
31 Scott's Wharf
Newport, RI

Harbour Shellfish Inc.
South County Trail
Exeter, RI

Pt. Judith Fishermen's Cooperative Assn.
Pt. Judith, RI

Rhode Island Fish Co.
515 South Water St.
Providence, RI

Saltesea Packing Co.
315 Geo. M. Cohan Blvd.
Providence, RI

Tallman and Mack Fish
Spring Wharf
Newport, RI

Wickford Shellfish Co.
67 Esmond Avenue
North Kingstown, RI

Yankee Seafood
Galilee, RI

Finn's Shellfish
Water Street
East Greenwich, RI

2. Marinas and Yacht Clubs

Warwick Cove Marina
2 Seminole Road
Warwick, RI

Apponaug Harbor Marina
Arnold's Neck Drive
Warwick, RI

East Greenwich Yacht Club
Water Street
East Greenwich, RI

3. Service Industries

East Greenwich Divers World
East Greenwich, RI

U.S. Weather Bureau
Green Airport
Warwick, RI

Northeast Marine Pilots
2001 Industrial Bank Bldg.
Providence, RI

R.I. Divers Assoc.
Elmwood Avenue
Providence, RI

Federal Pilot Assn.
282 Thames Street
Newport, RI

4. Supply Industries

New England Bilogical Assn.
Houston Avenue
Pt. Judith, RI

Acme Tackle Co., Inc.
69 Bucklin Street
Providence, RI

Bliss Marine Supply
Post Road
Warwick, RI

5. Boatbuilding and Sailmaking

Grumman Allied Industries
West Shore Road
Portsmouth, RI (683-0100)

Stanley's Boatyard
Barton Avenue
Barrington, RI

Thurston Sails, Inc.
406 Water Street
Warren, RI

Bristol Yacht Co.
Franklin Street
Bristol, RI

6. Heavy Industry

Naval Underwater Sound Lab
Newport, RI

General Dynamics
Electric Boat Division
Quonset, RI

Shipyard
Port of Providence
Providence, RI

Gulf Oil
Veterans Memorial Parkway
East Providence, RI

7. Scientific Study

Lafayette Trout Hatchery
Hatchery Road
North Kingstown, RI

Coastal Resources Center
URI School of Oceanography
Narragansett, RI

8. Salvage and Dredging

R.I. Dredge and Dock Co.
474-b Read Avenue
Warren, RI

B. Refer to Euel Gibbons, Stalking the Blue Eyed Scallop or Carolyn Kelley, Carolyn's Seafood Recipes, and prepare some seafood dishes in conjunction with the Home Economics Department.

UNIT XVI

MARINE GEOLOGY

I. BATHYMETRIC ANALYSIS

Objective:

To map the depth of a chosen area and compare with published depth charts.

Materials:

30 meters of line, compass, marine chart for specific area.

Procedure:

Make transects of the area chosen, spaced 30 meters apart. Site a reference point on shore and use a compass to stay on course. Determine water depth by using a weighted line about every 30 meters (fix each position by triangulation). Distances may be altered to suit.

In classroom at any time: using a chart of the area studied, mark out each depth from triangulation data and depth data. Note tide and date. Draw contour lines.

Compare your depth with those published. Be sure to correct your own values to low tide. Note date of your chart. Explain any differences you find.

II. BOTTOM MATERIALS ANALYSIS

Objective:

To map the bottom material type of a chosen area.

Materials:

Grab sampler, compass, marine chart of specific area.

Procedure:

Make transects of the area chosen, spaced 30 meters apart. Site a reference point on shore and use a compass to stay on course. Determine bottom type about every 30 meters (fix each position by triangulation). Distances may be altered to suit. The following classification is suggested: mud, sand, gravel, rock, shell.

In classroom at any time: using a chart of the area studied, mark the bottom type(s) for each point, using triangulation data. Note data. Draw contour lines.

III. USE OF THE GRAVITATIONAL CORE SAMPLER

Objective:

To learn to use the core sampler; to learn how to classify marine sediments; to learn to "read" the earth's history from the sample core (i.e. what do different sediment types indicate).

Materials:

Core sampler, extra clear plastic tubes, chemical test kit for calcium and silica.

Procedure:

On Water: Load the sampler with a tube and lower the sampler into the water. Record water depth and location. Raise sampler and recover plastic tube. Note colors of the layers, if any. (Colors will be noted later after core has dried, and a comparison made.) Be careful not to dislodge core from the tube. Tubes will be taken back to the lab for examination of cores.

In the Lab: Remove the core from the tube with a plunger and slice open before drying is complete. When dry compare colors of layers against color when wet. Chemically identify as many layers as your teacher chooses. For example, carry out the chemical tests for calcium and silica.

Questions:

1. What types of organisms are represented?
2. What does the stratification of the core indicate from the geological history standpoint?

Teacher Notes:

1. This corer cannot be used in sandy or gravel bottoms.
2. Reference - The Oceans, Sverdrup, et al.

SUPPLEMENTAL PROJECTS

I. BEACH, BAR, AND COASTAL MODELS USING A STREAM TABLE

Objective:

To investigate the effects of waves and longshore currents on sloping shores.

Materials:

Obtain or make a watertight tray optimum size .94 meters x 1.2 meters x 10 cm or larger. Tray can be made of marine plywood and sealed on inside with fiberglass or silicone sealant or tar.

For homemade construction, you will need: 2 lengths of 6.3 mm tubing,90 cm x 122 cm x 1.26 cm sheet of plywood (6PX waterproof plywood), 2 strips of plywood 10 cm x 94 cm x 1.9 cm, 2 strips of plywood 10 cm x 126 cm x 1.9 cm, rectangular block of wood approximately .5 cm, bucket of sand with variety of grain sizes, bucket to catch water runoff.

Nail 10 cm panels around edge of plywood sheet.

Drill hole in one end of stream table through 10 cm x 22.8 cm panel, large enough for 10 cm tubing to fit.

Prop up opposite end with block of wood and pour sand and water in at end.

Procedures:

1. Shoreline

   Make any way desired. Land (sand) - vary texture and show results of erosion.

   Move the fan to one side of the stream table and make waves near the shore.

   Sketch a top view of the waves approaching the shoreline.

   What happened to the shoreline? In five minutes? In ten minutes? In fifteen minutes?

   Look at the grains of sand in the table. Are all the grains where the waves washed onto the shore about the same size? Why?

2. Peninsula

   Scoop out the sand to make a bay and a peninsula in the shoreline.

   Repeat Exercise 1 for 5 minutes.

   How did waves affect the peninsula?

3. Tidal Flats

   What do tides do to gently sloping shorelines?

   What do tides do to steep shorelines?

Slowly raise the land end of the stream table 10 cm. When the water becomes still, slowly lower the table. Repeat this raising and lowering 10 times.

What happened to the sand when the table was raised and the tide went out (ebbed)?

What happened to the sand when the tide came in (flowed) as the table was lowered?

Sketch the changes in the shoreline after the 10 tides have ebbed and flowed.

Place some stones along the shoreline.

Create tides as above. What happened to the sand when the tide ebbed? What happened to the sand when the tide flowed?

Describe or sketch the changes in the shoreline after the 10 tides ebbed and flowed.

4. Coastal terraces

How are coastal terraces formed?

Set up the stream table with a bay and a headland.

Observe the shoreline for 5 minutes as waves wash against it. How did the straight section of coastline change? How did the bay coastline change? How did the headland coastline change?

Observe the new coastline as waves wash against it for 5 minutes. How did the straight section change? How did the bay change? How did the headland change?

II. PERCOLATION TEST

Purpose:

Determine the relative water content of different zones and elevations.

Procedure:

Remove top and bottom lid of a coffee can. Place the percolation can at 15 mm depth in the soil or sand being tested.

Rapidly fill the can with sea water and record the time it takes the water to completely percolate into the soil.

III. PARTICLE DISTRIBUTION ON SANDY BEACHES

Objective:

To study the relationship between particle size and elevation on several beaches.

Discussion:

In the development of a sandy beach the natural sorting out of the material which forms the beach causes certain sized particles to be left at the top of the slope (farthest from the water) while other sizes are moved toward the bottom (closest to the water). But, because of the constant movement of the material by tide and surf action of varying heights and force, there will be some material of virtually all sizes at any level along the slope of the beach.

In this exercise students will go to various beaches and sample sand from at least three spots -- high tide, mid tide, and low tide levels. They will sort them by particle size and determine if there is any relation between elevation on a beach and percent particle sizes found there. If so, why?

Materials:

Collect sand samples from three elevations on selected beaches. Note the slope of the beaches you work on. Use about a pint-sized can for each sample and try two-three beaches with slopes of different angles.

Procedure:

Place a moderate quantity of the sample in the top of the stack of sieves (do not attempt to fill the top sieve). Be sure the sieves are stacked in order of decreasing size of screen, the largest being on the top. Be sure the sand is dry.

Shake the sample through the sieves. Remove the material from each sieve (be careful to get all of it out of each) and place it on a piece of paper. Number the papers from one through five, with No. 1 being the largest and No. 5 the smallest.

Weigh each size sample carefully. Add the weights of the size samples in order to get the total weight. Calculate the percentage of the total sample represented by each size sample and record on data sheet.

Plot your results on a graph with the percent of total sample on the vertical axis and the particle size on the horizontal axis.

References:

Bascom, W. 1964. Waves and Beaches. Anchor Science Study Series, Doubleday, New York, N.Y.

IV. CORROSION RESISTANCE OF MATERIALS TO ENVIRONMENT

Objective:

To test the corrosion resistance of various materials.

Discussion:

The behavior of materials in a highly corrosive environment is well understood. The behavior of materials (coatings, specifically) in the invironment of space is much more subtle. The action of water vapor, trace amounts of various chemicals, ultraviolet radiation, and various gases can play a part in the degradation of a coating. The properties of these materials may change drastically after prolonged exposure. This laboratory exercise is open-ended and goes on for an extended period of time. Periodic inspection is required.

Materials:

corrosion test chambers
materials to be tested
salt water.

Procedure:

Put salt water in jar. Attach to stopper several strips of metal or rods. These may be coated with various test materials. Suspend the strips or rods in the water. Store jars in areas with different temperatures.

Students should be allowed to make their own combinations. They should note any changes which have occurred. Stress to them that this experiment takes time.

Record the following:

1. Salinity of test water.
2. Type of test material.
3. Date experiment began.

V. OCEAN FLOOR SPREADING

Objective:

To study how the ocean floor changes using a model.

Discussion:

The ocean floors are known to be moving slowly away from ridges like the

one that bisects the Atlantic Ocean (the Mid-Atlantic Ridge) as molten rock rises into the ridges to fill the gaps. When looking at the ridges, one observes they are not continuous; they are repeatedly offset to one side or the other. Furthermore, these offsets are joined by faults that lie parallel to the directions of sea floor motion. This exercise helps to explain the formation of these cracks in the earth's crust. The wax-tray method is taken to be an analogue of the earth's crust that floats on a relatively plastic region immediately beneath it.

Materials:

Paraffin, variable-speed fan, tray (metal), paddle

Procedure:

Melt paraffin (candle wax) in the tray. Place paddle in pan, about one-third of the way from the front (fan side). Turn on fan to cool wax. When surface film becomes solidified, pull paddle through it. This will compress the crust on one side of the pan and stretch it on the other. Watch the stretched side carefully. The crust will break into cracks perpendicular to the direction of stretching, and molten wax will well up into these cracks from below to form a new crust. Note that the cracks do not occur in one continuous line but in segments. The dragging created transform faults, linking offset segments and continuing well beyond them.

References:

"Continents Adrift," Scientific American.

Heezen, Bruce, and Ian MacGregor. 1972. "Riddles Chalked on the Ocean Floor," Saturday Review, Feb. 19.

Mathews, Samuel W. 1973. "This Changing Earth," National Geographic. January.

VI. SEDIMENT CORE ANALYSIS

Objective:

To learn how to determine geological history from a core sample.

Introduction:

The analysis of sediment core samples can give a picture of history. The geologist measures particle size and from this information determines how the sediments were deposited. They may arrive by current transport or just the steady accumulation of matter from the overlying water. The biological oceanographer is interested in sizes also, as this determines the kind of organisms living in the sediment. The water and organic content are also important for animal life since many animals feed on detritus (decaying organic matter).

The chemical oceanographer is interested in the composition of the sediments and what chemical reactions are occurring. The salinity of the sediments may also give clues to the past history.

General:

1. Once you have obtained your core samples you should draw them. These drawings should be accurate and to scale as this will be your only opportunity to see the core as it originally was. You can also use this drawing for future reference. Cut the core down the middle to expose the uncontaminated inside. Divide the core into samples choosing natural divisions if possible or equally spaced divisions if no layering is present. Identify each of these divisions with a symbol for reference.

2. Once you have competed the drawings, you should start your analysis immediately, First remove samples from 1/2 of the core for diatom analysis and place in jars labeled with the station and depth. Take samples at the divisions made above since there may be some change in the diatoms. Also save any interesting objects found in the core and put in labelled jars. Then use the remainder of this 1/2 sample to collect water for salinity.

	a.	water		d. salinity
1/2 sample:	b.	organic	1/2 sample:	e. pollen
	c.	sediment		f. diatom

Salinity of the entrapped water

1. Take your sample and wrap it in a fine piece of silk. Twist the ends so that the silk is tight around the sample Place the silk with the sample in a beaker. With a wood plunger you will be able to force the water out. Hold the beaker in such a manner that the mouth is facing down. Make sure you have a bottle underneath the beaker.

2. Once you have forced the water from your sample, either store it in a closed container or titrate immediately using prepared kit or hand method.

Water Content Analysis:

1. Remove about a 10 g. sample of the core and place in a previously weighed 50 ml. beaker.

2. Weigh the beaker and sample and determine the net weight.

3. Heat slowly in the beaker using a bunsen burner and dry the sample completely. Reweigh the beaker and dry sample and determine the amount of water lost and the percent of the original wet sample.

4. Save the dried sample in the beaker for the organic analysis.

Organic Analysis:

Hydrogen peroxide acts as an oxidizing agent and rapidly converts the organic materials into gaseous compounds which will bubble off and evaporate. The carbon becomes carbon dioxide, the hydrogen and oxygen form water vapor and the nitrogen forms ammonia gas.

1. Carefully mash the dried sample obtained in the water content analysis. Do not lose any sediment.

2. Add enough 30% hydrogen peroxide to cover the sample.

3. When the bubbles have stopped (about 1 day) dry carefully and re-weigh the sample. Calculate the lost weight which is the organic content.

4. Determine the percent this represents of the original sample.

Diatom Content Analysis:

1. Boil sample in a soap solution. This is to mix it up.

2. Once the sample is thoroughly mixed up, sieve it through a 149 micron sieve and then a 37 micron sieve.

3. Take the sample in the 37 micron sieve and transfer it to a vial. This can be done by washing the sieve with distilled water.

4. Once you have transferred the sample into a vial, shake it up. With an eyedropper, take some sample and put two drops on a slide.

5. Scan the slide with low power. To identify the diatoms, use high power and a text with a lot of drawings of diatoms.

Pollen Analysis:

1. Take your sample, about 10-15 grams and sieve it through a 149 micron sieve and then a 37 micron sieve.

2. Place the sample in a beaker and mix with 70-100 ml. HCl (20%). Let it sit for 3 days.

3. After 3 days pour off the HCl. Wash the sample with distilled water.

4. Mix the sample with 50 ml. HF (48%). NOTE: Hydrofluoric Acid is a very strong acid. Wear gloves. You must transfer the sample to a plastic container that can be sealed. HF will eat through glass. Add the HF after the sample is in plastic.

5. Let the sample sit in the HF for about 3 days. Pour off the HF (See disposal of HF below). Wash the sample with distilled water. Prepare slides.

DISPOSAL OF HYDROFLUORIC ACID:

Pour the HF into a bucket that is 1/2 full of water and has .45 kg of Boric Acid mixed in it. Let the HF sit in this bucket for a few days before pouring down the drain. The bucket must be plastic.

VII. MOVING DUNES ON A STREAM TABLE

Objective:

To study the effect of wind on sand dunes.

Discussion:

Hurricane Carol of 1954 caused great damage to Rhode Island's shoreline. Houses were torn apart. Large buildings were weakened by water washing the ground from under them. Roads were destroyed.

Why was damage from this storm so great? Many engineers said that man had removed some of the natural protection. This natural protection was in the form of sand dunes. When the sand dunes were smoothed out, the wind and high water had nothing to stop them. This caused more damage to property than there should have been.

What are sand dunes? How are they formed? These are questions man has to answer if he is to understand the oceans and their shores.

Sand is made up of small chips of rock. These chips are so small that they are easily carried by both wind and water. As the wind blows across the sand it picks up pieces of sand. A strong wind can carry a lot of sand. If the wind slows down it drops the sand. A dune may be formed in this manner.

Materials:

Large stream table or sand box.

Large box or sheet to catch sand.

Fan or vacuum cleaner (reversed) for wind source.

Procedure:

Make a number of hills and valleys in the steam table, keeping the land sloping upward toward the box.

Place a large cardboard box, open at one end, on the land portion of the stream table. Use fine, dry sand to represent sand dunes. Place a stone and a small bushy plant on the sand. Create wind by locating a fan at the end of the stream table, facing the open end of the box. Have students observe how the wind affects the dune shapes and locations. Ask the following questions:

What is happening to the sand on the front of the dune?

What is happening to the sand at the top of the dune?

What is happening to the sand on the far side of the dune?

What is happening to the sand around the stone?

What is happening to the sand around the bush?

Do sand dunes move?

Sketch a cross-section of a coastal terrace on the chalkboard. Ask students how such a coastal line might have been formed. Summarize all suggestions on the chalkboard and ask the class which suggestions seem most logical.

VIII. SEDIMENT TANK

The sediment tank simulates the formation of a delta as a river flows into the ocean or a lake and drops its sediment load. The sand is carried by the water flowing from the plastic tube until it meets a different energy environment - the ocean (the water level in the tank). The velocity of the river decreases as it enters the quiet water so that it can no longer carry heavy particles.

The delta slope is formed as particles settle out differently according to their weight. Where sand-laden streams flow into a still body of water three distinct beds form. The topset bed is a thin layer of sediments which fingers out over the delta surface. Thicker foreset beds accumulate on the face of the slope. The finest sediments remain in suspension for a time and are finally deposited beyond the slope in front of the advancing foreset beds. By means of the sediment tank students can observe the effect that stream velocity and particle size have sedimentation.

After the delta is formed wave erosion can be generated to erode it by reducing the water level below the delta surface and moving a ruler back and forth in the water.

How to Make a Sediment Tank:

1. Order 1/4" glass with ground edges cut to make an all-glass tank 7.5 cm x 20 cm x 120 cm. (If you plan to do the Sea Breeze or Ocean Circulation experiment, also order a glass lid 7.5 cm x 120 cm to lay across the top).

2. Put glass plates together with glass tape or masking tape.

3. Apply Silastic - silican cement made by GE along the inside seams of the plates. Smooth out so that there is a solid strip of Silastic along all seams of tank. Remove tape after about 8 hours when Silastic is thoroughly dried.

4. The water level can be adjusted by means of a siphon with a valve to control water flow.

Recommended reading:

The Life of Rivers and Streams, by Robert L. Usinger, McGraw-Hill, 1967.

Ecology of Inland Waters and Estuaries, pp. 35-38, by George K. Reid, Reynold Publishing Co., 1961.

Other Uses for Sediment Tank:

1. If you want to use the sediment tank as a wave tank, you should construct it of plexiglass and plexiglass cement solvent. Otherwise the alcohol and paint thinner would eventually dissolve the Silastic. Complete directions are given in Construction Details for Wave Machine.

2. Sea Breeze Demonstration
In many coastal areas particularly during the summer, the winds at the beach begin blowing from the sea during the morning. At night the wind direction will reverse. This demonstration illustrates how sea breezes work and emphasizes water's large heat capacity. One half of the bottom of the tank should have less than an inch of water; the other half is filled with crushed charcoal or a piece of styrofoam to simulate land. Provisions must be made to keep the charcoal and water separated by use of putty or clay. After the contents of the tank come to thermal equilibrium, two bottlecaps filled with ammonium hydroxide and hydrochloric acid respectively, are placed on the water side. The combined vapors from the bottle caps will provide a white tracer by which the air motions can be followed. The glass cover is put on the tank and a sunlamp, representing the sun, is lit above the tank. The charcoal "land" absorbs heat and warms quickly. The water because of its large heat capacity, does not warm as fast. This results in the cool dense air moving underneath the warm lighter air. Turn off the lamp and the direction of the winds reverse.

3. Ocean Circulation:
Fill the tank with water and lay the lid on top. Circulation can be stimulated by heating near the bottom at one side and cooling at the bottom on the other side. This will induce strong circulation; analogous to the fueling of the atmosphere. If the

tank is heated at the top on one side and cooled at the top on the opposite side a much weaker circulation is generated. This is a simplified analogy to the fueling of the vertical circulation of the ocean.

UNIT XVII

SPRING HERRING CENSUS

Objective:

To study some of the characteristics of herring (buckeyes or alewives) intercepted on their migration route from the ocean to their fresh water spawning grounds. We will want to determine each fish's sex, length, weight and attempt, randomly, the age of some specimens.

Materials:

dip net
spring balance
hand lens
forceps
meter stick

Locations:

1. Gilbert Stuart's Birthplace (Saunderstown)

2. Hamilton (Wickford)

3. Buckeye Brook (Warwick)

Procedure:

Determine the length of a specimen by measuring with a meter stick. Measure from the top of the nose to the middle of the caudal (tail) fin. Using the spring balance weigh the fish.

By gently squeezing the lower abdomen you will be able to express a small quantity of eggs (orange in color) or milt (milky white) in order to determine the sex of the specimen. If no gametes are expelled, the herring are returning to the sea. Dissection and inspection of gonads would prove this.

Using forceps remove a single scale and place in a numbered envelope. Record on Data Sheet.

Conclusion:

1. Using the annular ring method attempt to determine the ages of the fish.

2. Graph the data above for the length and weight.

3. What is the apparent age range of the fish?

4. Is there any distinction to be made between males and females with regard to length and weight? Explain.

UNIT XVIII

ECHINODERMS

Objective:

To observe the characteristics and external features of several representative Echinoderms.

Materials:

Live starfish, live sea urchins, petri dishes, fingerbowls, hand lens, stereo microscope, probe.

Project 1 - Study of a live starfish (Asterias vulgaris or forbesi)

Procedure 1 - Place a starfish in a petri dish and cover the animal with sea water. Watch the animal closely for several minutes. Describe the starfish as though you had never seen one before.

Procedure 2 - Study the aboral (side away from the mouth) side with the stereo microscope. Look for the spines. Each spine is surrounded by tiny pincer-like jaws called pedicellaria. Also between spines you may be able to see delicate finger-like gills. A tiny opening in the middle of the center section is the anus. The sieve plate or madreporite should be clearly visible as an orange spot located between a set of adjacent legs a short distance from the anus, located in the exact center of this side. You may be able to see tiny pores in the madreporite. At the end of each of the five arms is a tiny tentacle. At the ventral base of each tentacle is an eyespot. Touch a tentacle with your probe. Is there any reaction?

Procedure 3 - Turn the starfish over so that the oral (mouth) side is up. Locate the mouth in the center of the five arms. Running along the center of each arm from the central disk to the end is the ambulacral groove. Each groove has tube feet in it. How many rows? Watch the action of the tube feet. Describe how the starfish turns over. Sketch both sides of the starfish and return it to the bucket.

Project 2 - Sea Urchins

Procedure 1 - Place the sea urchin in a finger bowl and cover the animal with sea water. Observe the animal for several minutes and answer the following questions.

a. Where are the tube feet?

b. How are the feet arranged in each row?
c. How many rows of feet are there?
d. Do the spines move?
e. Are the spines sensitive to touch?
f. Is there a madreporite?
g. How does the animal move?

Procedure 2 - Turn the sea urchin over. Find the mouth and teeth. How many teeth?

Procedure 3 - Sketch and label your sea urchin. Then return it and the sea water to the bucket.

SUPPLEMENTAL PROJECTS

PROJECT: PEDICELLARIAE OF STARFISH AND SEA URCHINS

Discussion:

The body surfaces of sea urchins and starfish are covered by organs unique to echinoderms. These small, specialized, jaw-like structures known as pedicellariae are used to grasp and crush small organisms that attempt to settle on the animal's exterior surface. Thus, sea urchins and starfish are always clean and free of attached animals and plants.

Procedure:

Place a sea urchin under a stereomicroscope and look for small pinching organs located on the aboral surface between the spines. Take a strand of human hair and draw it across the surface of the sea urchin.

1. Was the hair grasped or pinched?

2. Place a sea urchin or starfish (with the aboral side facing down) on the back of your hand or on top of your head. Leave it there for a couple of minutes and then lift it away. Have the pedicellariae grasped any hairs?

3. Is it possible to induce pedicellariae to close in response to stimuli other than touch?

4. Can you derive another possible function of the pedicellariae?

5. Place a starfish and a sea urchin together in a small fenced off portion of the aquarium overnight. The following day observe the oral surface of the starfish for the presence of pedicellariae belonging to the sea urchin. What do you suppose happened during the night?

6. Should sea urchins and starfish be concerned about small organisms wanting to attach to them?

PROJECT: FEEDING STARFISH

Discussion:

There is some controversy whether starfish have sense organs which detect chemicals diffusing from the prey animal and then move toward the prey or whether prey location is by chance, the starfish ceasing to move only when it actually touches the prey. Perhaps the following exercise will resolve this question, at least in your mind.

Procedure:

Place starfish which have been starved for at least two weeks, at various distances from clams or mussels and observe the movements of the starfish. (Make sure the water is not stirred during this exercise.)

1. Is the movement of the starfish modified by the presence of the bivalves?

2. Do they show directed movement toward the bivalves? Do any of the behavioral modifications noted depend on the distance from the prey to the starfish?

3. Does the rate of movement of the starfish increase as it approaches the prey?

4. Repeat these observations using sponges soaked in clam or mussel juice as attractants instead of the living animals as attractants.

5. Do they appear to move faster? Slower? No difference.

Most starfish typically evert the cardiac stomach through their mouth and digest food outside their body. The everted cardiac stomach can be seen either by feeding a small piece of fish to a starfish attached to a glass wall of an aquarium or by rubbing a piece of food against the glass and then holding a starfish against the treated area until it attached. If the starfish has been starved for at least two weeks, the cardiac stomach should quickly evert. Is there an advantage to starfish in having the ability to digest food outside of its body? For further information see Supplemental Information.

PROJECT: GEOTAXIS IN STARFISH

Procedure:

Many species of starfish move up the walls of the aquarium and stay near the waterline. To determine whether gravity or any pulling force has

an effect on this behavior, stretch a rubberband around the central disc of an animal so that it is between the arms. Attach a small float to the rubberband such as a plastic bottle or cork. The upward pull should be of sufficient magnitude to float the animal when it is detached from the aquarium surface. Place the animal at mid-level on the wall of the aquarium and determine in which direction it moves. Run several trials.

What type of gravity response (positive or negative geotaxis) do starfish exhibit?

Is any direction preferred if the float neutralizes the weight of the animal in sea water?

Is there any advantage to the starfish in possessing such a response?

Explain:

To test the strength of adherence, remove a starfish from the aquarium and tie a string to one arm of the animal and to the other end attach a small spring scale. Place a sheet of plastic or cellophane wrap containing a 1/2" - 1" diameter hole over a glass plate and place the arm of the starfish over the hole so that it can attach a limited number of tube feet to the glass. Count the number of tube feet which have attached to the glass through the hole. Pull on the scale and note the force required to detach the animal from the glass. Divide the force required by the number of feet attached to obtain an index of attachment strength per tube foot. Try this for a number of specimens.

SPECIMEN	Force/no. of tube feet	=	Attachment strength per tube foot
1.			
2.			
3.			
4.			
5.			

PROJECT: SEA URCHIN - FERTILIZATION AND EARLY DEVELOPMENT

Introduction:

Keep specimens in the refrigerator or in a cool place in the room (60-70°F) until ready for the experiment. The number of urchins gathered is far in excess of the minimum number required for successful observation of fertilization and development. Each female will spawn one billion eggs and each male will eject one million sperm. Therefore, successful shedding of eggs by one female or the sperm from one male is sufficient for more demonstrations than the average class could perform. If the urchins are kept at about 34°F (0-2°C) they will survive for 3 days and still produce eggs and sperm, though the sooner they are used the greater will be the chances for success.

After reading these instructions, the teacher may choose to start the demonstration as early as possible on the day the students will be using eggs and sperm for their own experiments. You will note that a suspension of fertilized eggs may be prepared at different time intervals throughout the day with each suspension of eggs labeled with time of insemination so that various developmental stages may be observed throughout the school day. Some students in morning classes will want to return after school to observe the progress of cell development. NOTE that the first cleavage required 70-80 minutes after fertilization. Therefore, plan on inseminating an egg suspension in plenty of time for early classes to be able to observe the early cleavage stages of development.

Materials:

1. Filtered sea water
2. 30-50 sea urchins
3. Medicine dropper pipettes
4. Sterile petri plates
5. 2 five ml. syringes
6. 50 ml. 0.5 molar KCl solution
7. Microscopes
8. Graduated cylinders, 50 or 100 ml., CLEAN
9. Fingerbowls, flasks, or watch glass. All glassware should be as clean as possible.
10. CLEAN 250 ml. beakers.
11. As many CLEAN glass plates as needed for the number of urchins you intend to use at the time.

Procedure:

1. Arrange your glasware, syringes, graduates and beakers in a convenient working arrangement on your demonstration table. Place 25 ml. of sea water in several 250 ml. beakers in preparation for receiving eggs.

2. Fill the syringe with KCl solution and insert the needle of the syringe for its full length through the soft membrane surrounding the mouth of the urchin into its body cavity. Inject 1-2 ML. KCl solution. Then place the urchin upright on a clean glass plate. In a few minutes the female will begin to shed a "creamy yellow" mass of eggs or, if it is a male, a "milky white" mass of sperm. This will distinguish the sex of the animal.

3. When it is apparent that the urchin is spawning, <u>invert</u> the animal over the beaker so that the spines suspend it on the lip of the beaker. If a 250 ml. beaker is so large that the urchin is not suspended by the lip of the beaker, USE A SMALLER BEAKER OR FINGER BOWL. The geniopore of the urchin is located exactly opposite the mouth of the animal on the aboral surface (the top of the animal when it is sitting upright). When the urchin is INVERTED and held above the water in the beaker, the eggs will drain directly from the geniopore into the water below.

4. Should the urchin prove to be a male by ejecting the milky white sperm, lay it, inverted, in a sterile petri dish so that it will eject the sperm into the sterile, DRY petri dish. Keep the other half of the petri dish to use as a cover when storing the dry sperm for later use.

5. Prepare several dilute suspensions of EGGS by the following method: Place 5 drops of concentrated urchin eggs, as collected in #3 above, in 100 ml. of sea water in a beaker to make 100 ml. of dilute egg-sea water suspension. The number of such suspensions you prepare will depend upon your needs and the time limitations of your teaching day. These dilute suspensions will contain the eggs which will be fertilized throughout the day. Such a suspension of UNFERTILIZED eggs will keep up to 6 hours if refrigerated to 40°F. These eggs in dilute suspension will be ready for insemination.

6. Prepare a dilute suspension of sperm by the following method: Dilute <u>one</u> or <u>two</u> drops of DRY sperm (from the petri dish) to about 10 ml. volume of sea water. This dilute suspension of sperm will be used to inseminate eggs. DO NOT USE DILUTE PREPARATIONS OF SPERM MORE THAN 20-30 MINUTES OLD. Therefore, make up only as much DILUTE sperm suspension as you intend to use in that length of time. The dry sperm will remain viable for 24 hours if kept covered in a refrigerator.

7. OBSERVING EARLY DEVELOPMENT - Students may take one or two drops of the suspension of fertilized eggs to observe with high power magnification, but do not prolong the period of observation as excessive heating will occur on the microscope slides and the developing embros will die.

8. GENERAL EXAMINATION OF EMBRYOLOGICAL MATERIAL - Students will enjoy examining unfertilized eggs, fertilized eggs, and suspension of viable sperm and comparing their appearance and behavior.

PITFALLS AND GENERAL INFORMATION

Strongylocentrotus purpuratus Arbacia punctulata should be kept in insulated containers and chilled in a plastic bag. The urchins will survive up to three days if kept cold (32-36°F, 0-2°C) and moist. Do not submerge them in the water. Either place the urchins in a refrigerator, if they are to be kept on hand, or provide additional ice in the chests. Sea urchins will not survive in fresh water; therefore, avoid direct contact of these animals with ice or the melt therefrom.

Male urchins may vary in the potency of their sperm; therefore, either screen several males to discover the male with highest potency or pool the gametes from several males in the preparation of the dilute suspension of sperm. As indicated above, do not use dilute preparations of sperm more than 20-30 minutes old.

Students in each class may care to examine and compare unfertilized eggs, fertilized eggs at various stages of development, and suspensions of sperm; therefore, according to class schedules, the following timetable may be modified to meet individual requirements.

To provide eggs at advanced stages of development, prepare and fertilize at least one suspension of eggs in advance of the class meeting. Eggs at the stage of first cleavage should be inseminated at least 70 minutes in advance of examination. The sequence of development for S. purpuratus (Lytechinus pictus follows at about the same rate) is reported as follows:

TIMETABLE

Formation of fertilization membrane	2 - 5 min.
First cleavage	60 - 70 min.
4-8 cells	4 - 5 hours
16-32 cells	6 hours
Early blastula	9 hours
Blastula	11 hours
Late gastrula	32 hours
Pluteus	48 - 68 hours

Rate of development varies with temperature, but do not exceed 70°F.

ADDITIONAL INFORMATION

Students may find that the inseminated egg material may tend to dry out on the microscope slide. If so, sealed "WHOLE MOUNTS" should be made. It is perfectly easy to seal a temporary mount with petroleum jelly, "Vaseline", provided that the jelly is applied to the slide and not to the cover slip.

Have each student sketch on a piece of white card, or paper, a square slightly smaller than the size of the coverslip to be used. Circles can

also be used but are more difficult to handle. Now lay a clean slide on top of the card so that the square is roughly centered and trace the outline of the square on the slide with petrolatum picked up on a toothpick. Set the prepared slides on one side. Take a clean coverslip and place a small drop containing 3-5 urchin eggs in the center. To avoid disappointing the student, it is often well for the teacher to check with a hand lens that the eggs are properly placed. Lay a couple of strands of thin nylon or other support on the coverslip, then invert the slide so that the vaseline square is underneath, and lower this carefully onto the coverslip so that the square of vaseline is centered over the coverslip. Press down carefully, holding the slide by the ends, until the vaseline is seen to be in contact all the way around. It is much better to have a small drop, the edges of which, even when the slide is pressed down, do not reach the edges of the coverslip, than to have too big a drop. An air space round the edges of the drop, provided the coverslip is adequately supported and sealed, provides a plentiful supply of oxygen.

ALTERNATE METHOD OF COLLECTING EGGS AND SPERM:

If the urchins fail to shed eggs or milt or if they shed them very slowly, you may want to try an alternate method of collecting eggs and sperm.

REMOVAL OF GONADS:

Place the urchin oral slide up and cut around the animal's shell with a scissors at about its widest circumference. Remove the upper part of the shell.

The gonads should be visible. The testes are white and the ovaries are yellow-orange. Snip the ovaries at the duct and place in a culture bowl about one-fourth full of fresh sea water. Allow the ovaries to stand in the bowl for about five munutes so that the ripe eggs may flow out of the ovaries. Place a piece of cheese cloth over the culture bowl and pour the egg suspension through this into another bowl of fresh sea water. If the eggs are to be kept for several hours, place a thin layer of eggs in a finger bowl, keeping the bowl covered so that little evaporation is allowed to take place.

When the testes are removed, place in a clean, dry finger bowl and keep covered. When ready to use, enough sea water is added to wash out the sperm in the same way that eggs were washed out of the ovaries. REMEMBER THAT SPERM WILL LIVE FOR ONLY ABOUT 20 MINUTES AFTER BEING MIXED WITH SEA WATER. Therefore, it may be desired to use only a portion of the testes to mix with sea water so that sperm can be removed. If such is the case, cut off a portion of the testes and extract the sperm from it, leaving the rest of the testes in the "DRY" finger bowl.

SUPPLEMENTAL INFORMATION

PHYLUM: ECHINODERMATA (ECHINODERMS)

I. Phylum: Echinodermata (Echinoderms) (Spiny-skinned)

A. Characteristics of the Phylum: Echinodermata

1. All are marine

2. None are known to be parasitic.

3. Spiny exoskeleton is composed, in some cases, of calcareous plates.

4. Bilaterally symmetrical when young; radially symmetrical when adult.

5. Most forms possess a water vascular system with tube feet.

6. They have great regeneration power.

7. Most of the members of this group are fossils. Relatively few forms exist today compared to the ancient past.

8. They do not resemble any other phylum of invertebrates. There is good reason to believe that they are more closely related to the vertebrates than to any other invertebrate phylum.

B. Example 1: Starfish (The common types found in this area are Asterias forbesi and Henricia sanguinolenta (Blood starfish)

1. The skeleton

a. Is made up of calcareous plates.

b. The spines are short and blunt. Around their bases are many whitish modified spines called pedicellariae.

c. The pedicellariae function as little jaws, which, when irritated may be opened and closed by several sets of muscles. Their function is to prevent debris and small organisms from collecting on the surface, to capture food and to protect the dermal branchiae (skin gills).

d. The dermal branchiae look like a soft furry substance on the aboral surface of the ray (arm). This appearance is

caused by the outpouching of the thin linings of the body cavity through minute openings in the skeleton. The dermal branchiae are covered with cilia on both the inside and outside. The external cilia keep a current of oxygenated water passing over the branchiae on the outside; and the internal cilia cause the body fluid to flow out into the dermal branchiae. While the body fluid is in the dermal branchiae, an exchange of oxygen and carbon dioxide takes place as it does in our own lungs when blood flows past the tiny air sacs in them.

2. The side that the mouth is on is called the oral side.

3. Tube feet are found on the oral side of each ray (arm).

4. There is an eye spot at the tip of each arm on the oral side.

5. The water vascular system:

   a. Water enters the madreporite (sieve) plate and passes through the stone canal, ring canal, radial canal, transverse canal, and to the tube foot. (The madreporite plate is on aboral side.)

   b. When the starfish presses its tube feet against an object and forces water out of its canals, the feet grip the object firmly by means of suction. Return of water to the canals releases the grip.

6. How starfish get food and the digestive system.

   a. The starfish arches over its prey with the rays bent downward. The valves of the clam or oyster are gripped firmly by the tube feet, and at the same time a steady pull is exerted.

   b. Then the starfish pushes the oral portion of its stomach out from its mouth and squeezes it through the tiny opening between the mollusk's valves.

   c. The stomach, which is inside out, secretes enzymes that digest the tissues of the clam or oyster.

   d. After digestion is completed by the other parts of the digestive system, the stomach is retracted and the starfish moves away leaving the empty shell of its prey behind.

   e. The mouth opens into a very short esophagus, which leads into the stomach.

   f. The stomach consists of two parts - a larger oral portion (the part that comes out) and a smaller phyloric portion, which does not come out.

g. From the phyloric portion of the stomach, a tube passes into each ray, then divides into 2 branches called the phyloric or hepatic ceca.

h. Above the phyloric portion of the stomach is a slender intestine, which opens to the outside through the anus.

i. Two branches pouches, brown in color, arise from the intestines. These are known as intestinal ceca.

j. Food of the starfish consists of fish, oysters, mussels, barnacles, clams, snails, worms, crustaceans, etc.

7. Circulatory System

a. The fluid in the coelom (body cavity) is kept in motion by cilia. This fluid, (you may call it blood, if you wish) is kept in motion by cilia. It carries the absorbed food to all parts of the body. Note: Cilia line the body cavity.

b. Thus, there is not definite circulatory equipment such as heart, blood vessels, etc.

8. Excretory System

a. Excretion (the process of removing liquid nitrogen wastes that result in the burning of food in the individual cells) is accomplished by ameba-like cells called amoebocytes which are present in the body fluid.

b. The amoebocytes carry the liquid nitrogen wastes outside of the body walls through the dermal branchiae.

9. Nervous System

a. Many nerve cells lie among the epidermal cells (ectoderm).

b. In each arm near the radial canals, there is a radial nerve cord. The radial nerve cord of each arm unites with an oral nerve ring, which encircles the mouth.

10. Reproduction

a. Sexes are separate.

b. The reproductive organs are branched structures. There are two at the base of each arm.

c. The female has been known to release as many as 2½ million eggs in 2 hours, and 200 million eggs may be liberated in a season.

d. Eggs are fertilized in the water and develop into a larva called a bipinnaria larva, which has bilateral symmetry. It is worm-like in appearance and free swimming.

e. After swimming for a period of time, it settles to the ground and gradually changes into an adult with radial symmetry.

f. Starfish have remarkable ability to regenerate A single arm with part of the disk will regenerate an entire body. If an arm is injured, it is usually cast off.

g. Recent investigations indicate that starfish increase and decrease in 14-year cycles:

1943	peak population
1950	small population
1957	peak population
1964	small population
1971	peak population
1978	small population (expected)

C. Example 2: Brittle Stars (sea snakes (snake star)

1. The name, "brittle star", is derived from the fact that these animals break off their arms when they become injured.

2. They have no pedicellariae.

3. The madreporite plate is on the oral side.

D. Example 3: Sea urchins (Strongylocentrotus droebachiensis) are found on the Atlantic Coast. The purple sea urchin, Arbacia punctulata is quite common around here.)

1. The skeleton of a sea urchin is called a test.

2. Food is ingested by means of a complicated structure known as Aristotle's lantern.

3. Their tube feet have suckers.

4. The spines of some tropical sea urchins are poisonous.

5. They have three-jawed pedicellariae.

E. Example 4: Sand Dollars (Sea Biscuits) (Echinarchnius parma is common in this area.)

1. Flat relatives of the sea urchins with the moveable spines greatly reduced in size.

2. They live in deeper water, half buried in sand, feeding on organic material and plankton.

3. Cilia on the spines move the food particles until they are trapped by mucus around the spines and are pushed into the mouth of the animal.

4. A large Pacific starfish feeds on sand dollars.

F. Example 5: Sea cucumbers (Thyone briareus is very common.)

1. 10 to 30 tube feet surrounding the mouth which serves as tentacles for procuring food.

2. No rays, spines, or pedicellariae.

G. Example 6: Sea lilies (feather stars)

1. These belong to a group called crinoids.

2. They are attached to the sea bottom by a long jointed stalk.

3. The rays, 5 to 10 in number are often branched near the base and bear smaller branches called pinnules along the sides, giving them a feathery appearance.

4. The tube feet have no suckers.

UNIT XIX

SUPPLEMENTAL ACTIVITIES

PROJECT: MARINE MICROBIOLOGY - AGAR DIGESTERS

Objective:

To demonstrate on a marine alga the presence of bacteria which utilizes agar as a food source, and thus to emphasize the great variety of materials which can support bacteria.

Discussion:

Gelatin is not a good medium on which to grow microorganisms, for many of them digest it by means of an enzyme, gelatinase. In part, use of agar solves this problem. Agar also has other valuable characteristics for the growth of microorganisms. However, in this exercise we will learn that even agar can be digested by certain bacteria. Thus, we can see that microorganisms use a wide variety of substances from which to get nourishment.

Agar is obtained from seaweed. In the red algae, agar is a carbohydrate secretion which adds to the cell wall material of the algae. Because bacteria are present everywhere in the sea in fantastic numbers -- from inside marine organisms to ocean depths of several miles -- and because the food sources in the sea are limited, we would expect to find bacteria preying upon every conceivable type of organism, including algae.

Materials:

Red algae which feel slimy to the touch
Marine agar digester medium
2 sterile petri dishes
Sterile knife or forceps
Inoculating loop
Iodine
Test tube
Sterile sea water

Procedure:

Before Class:

Pour autoclaved marine agar digester medium into two sterile petri dishes per team and allow agar to harden for one day.

In Class:

With a sterile knife or forceps, scrape off a layer of scum from the

surface of red alga and shake the scum into a test tube containing 10 ml. of sterile sea water.

Mix well and, with the inoculating loop, streak a few drops of this suspension over one of the agar plates.

Incubate the culture dish in a humid atmosphere (a plate of water next to the dishes will supply this) at a constant temperature.

Check the dish daily and record the growth observed. Count the number of colonies present.

Check the surface of the agar carefully. When little depressions or pits appear in the agar, remove some of the material from the pit by means of a sterile inoculating loop and streak on a second agar plate. This will enable you to get a pure culture of the agar-digesting bacterium.

Observe the growth of the agar-digesting bacterium in the second dish. Count the number of colonies in the second dish.

Flood one pitted plate with a layer of iodine solution. Pour off the excess and note the results.

Questions:

1. Do the digested pits contain liquid or are they dry?
2. Is the agar in the pits just softened or is it actually consumed?
3. In the iodine-covered plate, what is the color of the plate as a whole?
4. What is the color of the area around the pits?
5. What does this iodine test tell you about agar?
6. What does it tell you about the bacterial activity?
7. Assuming that there are millions of bacteria present in the colony you see as a dot on the agar plate, why would you expect to get a pure culture by touching the inoculating loop to one dot and spreading it on another plate?
8. Why would one look for an agar-digesting bacterium on red algae?
9. Check the formula for marine agar-digester medium. In this medium, what is the source of nitrogen?
10. What is the source of carbon?
11. What is the source of inorganic salts?
12. In the sea, what do bacteria use as sources of carbon and nitrogen?

NOTES FOR THE TEACHER:

Time: 40-50 minutes, plus observation time over the following week.

The purpose of the exercise is to demonstrate bacteria on a marine alga and to emphasize the variety of food sources utilized by bacteria.

Special Instructions

This exercise is designed for areas which have easy access to a marine environment and allows this environment to be used for the introduction and demonstration of bacteria. In such areas, it can serve as a substitute for exercises dealing with terrestrial bacteria.

The algae selected should be slimy to the touch. This usually indicates that agar digestion is in progress.

The streak-plate culture method is suggested because there is no strict time or temperature limitation for the streak-plate method, and beginning students frequently have difficulty with the pour-plate method.

Techniques

The following formula for agar-digester medium should be placed on the board, so that the students can answer the questions in this exercise.

1 liter sterile sea water
1g ammonium chloride
15g agar
.5g galactose

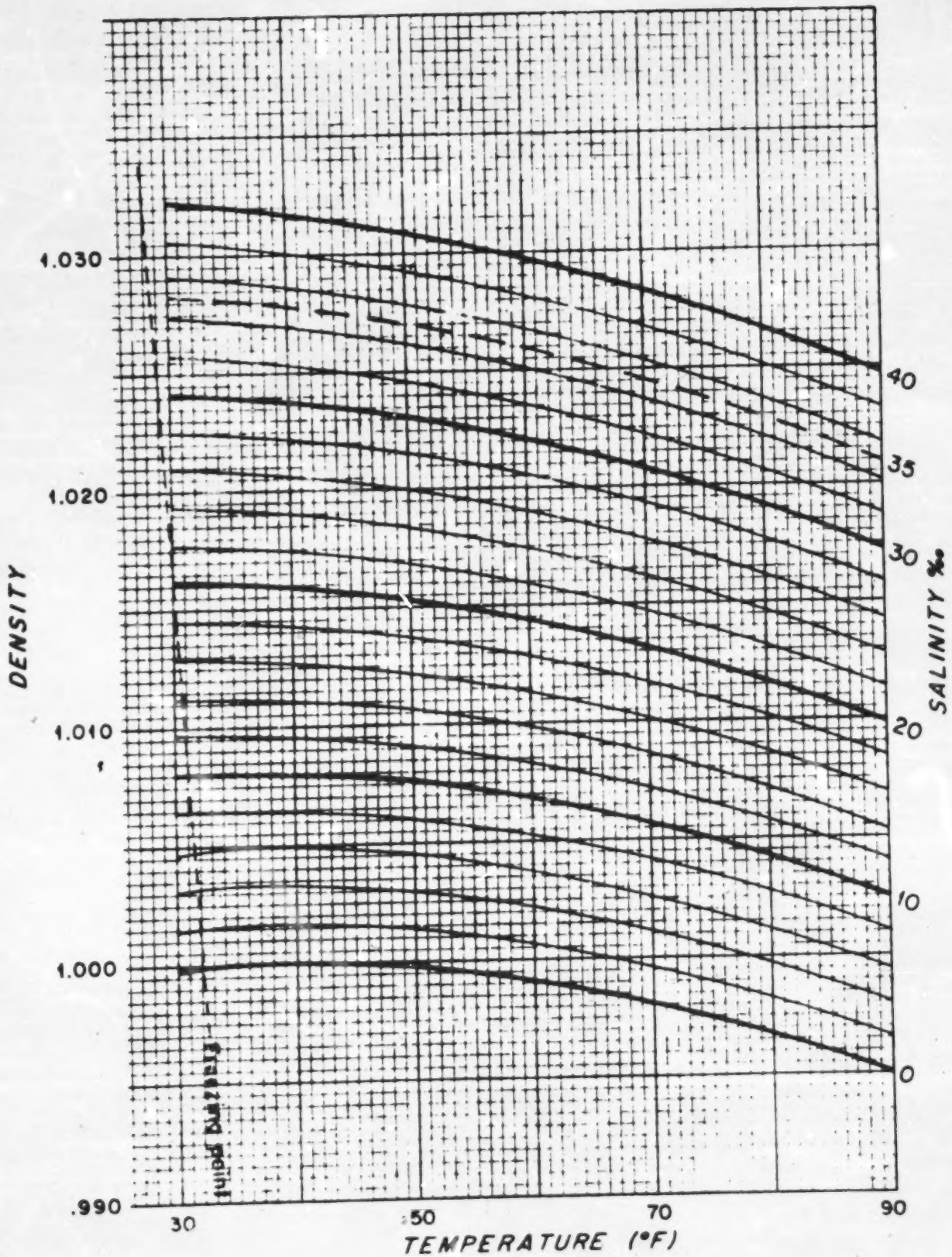
In the streaking, students should be cautioned not to break the surface of the agar.

Suggested Questions

1. Why did we look for an agar-digesting bacterium on red algae?

2. In the red alga, agar is a carbohydrate secretion which adds to the dead material in the cell wall. Is the bacterium studied necessarily a parasite on the alga?

3. Would you expect to find this bacterium on land?


1.030
1.020
1.010
1.000
.990
DENSITY
30
50
70
90
TEMPERATURE (°F)
40
35
30
20
10
0
SALINITY ‰

Temperature Conversion Table

To use the table, look for the temperature reading you have in the middle column. If the reading you have is in degrees Centigrade, read the Fahrenheit equivalent in the right hand column. If the reading you have is in degrees Fahrenheit, read the Centigrade equivalent in the left hand column.

-460.4 to 0			0 to 100						100 to 1000						1000 to 2000					
C		F	C		F	C		F	C		F	C		F	C		F	C		F
-273	-459.4		-17.8	0	32	10.0	50	122.0	38	100	212	260	500	932	538	1000	1832	816	1500	2732
-268	-450		-17.2	1	33.8	10.6	51	123.8	43	110	230	266	510	950	543	1010	1850	821	1510	2750
-262	-440		-16.7	2	35.6	11.1	52	125.6	49	120	248	271	520	968	549	1020	1868	827	1520	2768
-257	-430		-16.1	3	37.4	11.7	53	127.4	54	130	266	277	530	986	554	1030	1886	832	1530	2786
-251	-420		-15.6	4	39.2	12.2	54	129.2	60	140	284	282	540	1004	560	1040	1904	838	1540	2804
-246	-410		-15.0	5	41.0	12.8	55	131.0	66	150	302	288	550	1022	566	1050	1922	843	1550	2822
-240	-400		-14.4	6	42.8	13.3	56	132.8	71	160	320	293	560	1040	571	1060	1940	849	1560	2840
-234	-390		-13.9	7	44.6	13.9	57	134.6	77	170	338	299	570	1058	577	1070	1958	854	1570	2858
-229	-380		-13.3	8	46.4	14.4	58	136.4	82	180	356	304	580	1076	582	1080	1976	860	1580	2876
-223	-370		-12.8	9	48.2	15.0	59	138.2	88	190	374	310	590	1094	588	1090	1994	866	1590	2894
-218	-360		-12.2	10	50.0	15.6	60	140.0	93	200	392	316	600	1112	593	1100	2012	871	1600	2912
-212	-350		-11.7	11	51.8	16.1	61	141.8	99	210	410	321	610	1130	599	1110	2030	877	1610	2930
-207	-340		-11.1	12	53.6	16.7	62	143.6	100	212	413.6	327	620	1148	604	1120	2048	882	1620	2948
-201	-330		-10.6	13	55.4	17.2	63	145.4	104	220	428	332	630	1166	610	1130	2066	888	1630	2966
-196	-320		-10.0	14	57.2	17.8	64	147.2	110	230	446	338	640	1184	616	1140	2084	893	1640	2984
-190	-310		- 9.4	15	59.0	18.3	65	149.0	116	240	464	343	650	1202	621	1150	2102	899	1650	3002
-184	-300		- 8.9	16	60.8	18.9	66	150.8	121	250	482	349	660	1220	627	1160	2120	904	1660	3020
-179	-290		- 8.3	17	62.6	19.4	67	152.6	127	260	500	354	670	1238	632	1170	2138	910	1670	3038
-173	-280		- 7.8	18	64.4	20.0	68	154.4	132	270	518	360	680	1256	638	1180	2156	916	1680	3056
-169	-273	-459.4	- 7.2	19	66.2	20.6	69	156.2	138	280	536	366	690	1274	643	1190	2174	921	1690	3074
-168	-270	-454	- 6.7	20	68.0	21.1	70	158.0	143	290	554	371	700	1292	649	1200	2192	927	1700	3092
-162	-260	-436	- 6.1	21	69.8	21.7	71	159.8	149	300	572	377	710	1310	654	1210	2210	932	1710	3110
-157	-250	-418	- 5.6	22	71.6	22.2	72	161.6	154	310	590	382	720	1328	660	1220	2228	938	1720	3128
-151	-240	-400	- 5.0	23	73.4	22.8	73	153.4	160	320	608	388	730	1346	666	1230	2246	943	1730	3146
-146	-230	-382	- 4.4	24	75.2	23.3	74	165.2	166	330	626	393	740	1364	671	1240	2264	949	1740	3164
-140	-220	-364	- 3.9	25	77.0	23.9	75	167.0	171	340	644	399	750	1382	677	1250	2282	954	1750	3182
-134	-210	-346	- 3.3	26	78.8	24.4	76	168.8	177	350	662	404	760	1400	682	1260	2300	960	1760	3200
-129	-200	-328	- 2.8	27	80.6	25.0	77	170.6	182	360	680	410	770	1418	688	1270	2318	966	1770	3218
-123	-190	-310	- 2.2	28	82.4	25.6	78	172.4	188	370	698	416	780	1436	693	1280	2336	971	1780	3236
-118	-180	-292	- 1.7	29	84.2	26.1	79	174.2	193	380	716	421	790	1454	699	1290	2354	977	1790	3254
-112	-170	-274	- 1.1	30	86.0	26.7	80	176.0	199	390	734	427	800	1472	704	1300	2372	982	1800	3272
-107	-160	-256	- 0.6	31	87.8	27.2	81	177.8	204	400	752	432	810	1490	710	1310	2390	988	1810	3290
-101	-150	-238	0.0	32	89.6	27.8	82	179.6	210	410	770	438	820	1508	716	1320	2408	993	1820	3308
- 96	-140	-220	0.6	33	91.4	28.3	83	181.4	216	420	788	443	830	1526	721	1330	2426	999	1830	3326
- 90	-130	-202	1.1	34	93.2	28.9	84	183.2	221	430	806	449	840	1544	727	1340	2444	1004	1840	3344
- 84	-120	-184	1.7	35	95.0	29.4	85	185.0	227	440	824	454	850	1562	732	1350	2462	1010	1850	3362
- 79	-110	-166	2.2	36	96.8	30.0	86	186.8	232	450	842	460	860	1580	738	1360	2480	1016	1860	3380
- 73	-100	-148	2.8	37	98.6	30.6	87	188.6	238	460	860	466	870	1598	743	1370	2498	1021	1870	3398
- 68	- 90	-130	3.3	38	100.4	31.1	88	190.4	243	470	878	471	880	1616	749	1380	2516	1027	1880	3416
- 62	- 80	-112	3.9	39	102.2	31.7	89	192.2	249	480	896	477	890	1634	754	1390	2534	1032	1890	3434
- 57	- 70	- 94	4.4	40	104.0	32.2	90	194.0	254	490	914	482	900	1652	760	1400	2552	1038	1900	3452
- 51	- 60	- 76	5.0	41	105.8	32.8	91	195.8				488	910	1670	766	1410	2570	1043	1910	3470
- 46	- 50	- 58	5.6	42	107.6	33.3	92	197.6				493	920	1688	771	1420	2588	1049	1920	3488
- 40	- 40	- 40	6.1	43	109.4	33.9	93	199.4				499	930	1706	777	1430	2606	1054	1930	3506
- 34	- 30	- 22	6.7	44	111.2	34.4	94	201.2				504	940	1724	782	1440	2624	1060	1940	3524
- 29	- 20	- 4	7.2	45	113.0	35.0	95	203.0				510	950	1742	788	1450	2642	1066	1950	3542
- 23	- 10	+ 14	7.8	46	114.8	35.6	96	204.8				516	960	1760	793	1460	2660	1071	1960	3560
- 17.8	- 0	+ 32	8.3	47	116.6	36.1	97	206.6				521	970	1778	799	1470	2678	1077	1970	3578
			8.9	48	118.4	36.7	98	208.4				527	980	1796	804	1480	2696	1082	1980	3596
			9.4	49	120.2	37.2	99	210.2				532	990	1814	810	1490	2714	1088	1990	3614
						37.8	100	212.0				538	1000	1832				1093	2000	3632

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